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The Effects of Fluid Milk in Attenuating Postprandial Hyperglycemia and Hypertriglyceridemia

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The Effects of Fluid Milk in Attenuating Postprandial Hyperglycemia and Hypertriglyceridemia

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Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

December, 2016

Dedication

I dedicate this dissertation with profound gratitude to my parents, April and Dewey, for their unconditional love and support and for teaching me to do what I love with commitment and hard work. And to my brilliant husband, Brian, thank you for being a constant source of support, encouragement, and sound science. Thank you for sharing your life with me.

Acknowledgements

With sincere appreciation, I would like to thank my advisor and mentor, Dr. Hirofumi Tanaka, for his endless support and guidance throughout my doctoral studies. I am grateful for every opportunity, experience, and responsibility that has contributed to my development and growth as a scientist and academic. Most importantly, thank you for all of the time and effort you have committed to helping me succeed. It has been a privilege to work for and learn from you.

I am grateful to the members of my dissertation committee, Dr. Ed Coyle, Dr. Audrey Stone, and Dr. Jamie Davis. Experts in the field of metabolism, cardiovascular, and nutrition research, your input and guidance throughout my doctoral studies has been invaluable and I am grateful to have had the opportunity to learn from you. In addition to my committee members, I would like to thank the faculty and staff that I have worked with in the Kinesiology and Health Education, Nutritional Sciences, and Biological Sciences departments.

My dissertation work was funded by an external source and I would like to gratefully acknowledge the Dairy Research Institute for their support.

Thank you to all of my lab mates from the Cardiovascular Aging and Research Laboratory: Evan Pasha, Jitanan (Nui) Laosiripisan, Dan Machin, Mohammed Alkatan, Stephanie LaPierre, Brett Baker, Jisok Lim, Alex Kraus, Wes Geary, Stephen Roy, Wonil Park, Jeff Baker, Amanda Akkari, Raymond Cossio, Mohamed Elmoghany, Ahmed

Elmenshawy, Laura Delfausse, Rodrigo Ferrari, Jared Eaves, and Ichiro Hayashi. It was a pleasure to work with such talented colleagues and a privilege to earn your friendships. I wish you all the best of luck in your future careers and look forward to celebrating your inevitable successes.

Thank you to all of my friends and family for your love and support: Ruth, Tom, and Tran Hennessy; Don, Cricket, and Sydney Leary; Jen Ikemoto; Sonie Pearman; Christie Saenz; Dani Ashak; Amanda Reat; Jordan Patik, and The DeVallance Family. To my siblings, Rachel, Joel, and Alex, and my parents, April and Dewey: you are my best friends and fiercest allies. Thank you for cushioning every disappointment, celebrating every success, and always believing in me. Most importantly, to my husband, Brian Leary, without whom this dissertation could not have happened. Brian, every single thing I do is better because you are a part of it. Especially this dissertation.

The Effects of Fluid Milk in Attenuating Postprandial Hyperglycemia and Hypertriglyceridemia

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The University of Texas at Austin, 2016

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The increased risk of cardiovascular disease (CVD) and vascular dysfunction that is associated with postprandial hyperglycemia and hypertriglyceridemia may be reduced with consumption of non-fat dairy products. Due to the insulinotropic effect of milk-derived proteins, postprandial hyperglycemia has been shown to be reduced with the addition of dairy products. The purpose of Study 1 was to determine if one serving of non-fat milk added to an oral glucose tolerance test (OGTT) could attenuate postprandial hyperglycemia in individuals with elevated android adiposity independent of the effects of the milk's protein content and whether these improvements would be associated with metabolic and/or peripheral hemodynamic effects. In this placebo controlled, randomized, crossover experimental study, twenty-nine overweight/obese adults consumed an OGTT combined with either non-fat milk or a placebo control. In the whole sample, blood glucose and insulin concentrations increased over time in both trials with no differences between trials. When the subjects were divided into tertiles of android

body fat, the highest tertile displayed attenuated hyperglycemic responses as well as improvements in flow-mediated dilation during the milk intervention.

Repeated exposure to elevated postprandial triglycerides, as seen with typical Western diets, contributes to atherosclerosis and vascular disease. The purpose of Study 2 was to determine if a single serving of non-fat milk added to a high fat meal could attenuate postprandial hypertriglyceridemia in individuals who consume a high fat diet. In this placebo controlled, randomized, crossover experimental study, thirty overweight/obese adults consumed a high fat meal combined with either non-fat milk, carbohydrate control, or caloric control. In the whole sample, plasma triglycerides increased over time with no differences between interventions. When participants were ranked and divided based on habitual dietary fat intake, the high fat diet group exhibited reduced triglycerides when supplemented with non-fat milk. No differences in hemodynamic measures (brachial flow-mediated dilation and femoral vascular conductance) were observed between the milk and caloric control trials for either the low fat or high fat diet groups.

Taken together, the results indicate that a single serving of non-fat milk may attenuate acute hyperglycemia and hypertriglyceridemia in individuals that present with specific risk factors for CVD.

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GENERAL INTRODUCTION

While often referred to as a single entity, Metabolic Syndrome (MetS) is actually a constellation of interrelated metabolic risk factors that promote atherosclerotic cardiovascular disease (CVD) and type 2 diabetes mellitus [1, 2]. Two predominant risk factors contributing to MetS are insulin resistance and atherosclerosis; the cause and effect of both are intricately involved in postprandial metabolism [3-5]. Insulin resistance, a recognized hallmark feature of abnormal metabolic function, is characterized by excessive insulin secretion and/or impaired insulin signaling [6, 7]. Abnormal insulin response, including insulin resistance, leads to metabolic disturbances, including hyperglycemia, hyperinsulinemia, and hypertriglyceridemia that are maintained in a perpetual cycle of metabolic dysfunction [7].

Atherosclerosis is a form of chronic inflammation of the blood vessel walls that can lead to ischemia, end-organ damage, stroke, or myocardial infarction. The initial insult is the translocation of an LDL cholesterol molecule into the sub-endothelial space causing damage to the endothelium, the innermost lining of a blood vessel. This incites an inflammation and immune response that leads to invagination of the vessel lumen, resulting in reduced blood flow [1, 8, 9]. Eventually, the endothelium undergoes phenotypical alterations to this non-adaptive state known as “endothelial dysfunction.” Indeed, endothelial dysfunction is evident prior to any clinical symptoms associated with atherosclerosis and has been shown to be associated with an increased risk of CVD [10-12].

Traditionally, fasting plasma glucose and triglyceride levels have also been used as clinical determinants of CVD. However, as most individuals spend the majority of their time in a postprandial state, acute responses in plasma glucose and triglycerides following a meal have been shown to be better predictors of relative CVD risk [13-15]. Epidemiological data indicate that two-

hour glucose concentration measured during an oral glucose tolerance test (OGTT) is an independent predictor of CVD risk, whereas fasting glucose is not, and postprandial triglycerides are better predictors of atherosclerosis and coronary artery disease (CAD) than fasting levels [16-18].

The pathogenesis of both insulin resistance and atherosclerosis has been attributed to acute states of hyperglycemia and hypertriglyceridemia. Increases in postprandial plasma glucose or triglyceride concentrations following a high carbohydrate or high fat meal contribute to a pro-atherogenic metabolic state [19-21]. Moreover, both postprandial lipemia and hyperglycemia are associated with reduced vascular reactivity and impaired endothelial function [22, 23]. Importantly, while the exact mechanisms have yet to be described, the relation between postprandial metabolic impairments and endothelial dysfunction has been firmly established.

Epidemiological studies suggest that chronically high consumption of milk and dairy products are associated with a reduced risk of diabetes and CVD and are inversely associated with MetS [24-28]. The addition of dairy products to a meal high in carbohydrates may potentially lessen the risk of diabetes through reductions in postprandial glucose and triglycerides [29-33]. Several bioactive compounds found in dairy may possess functional properties although the identification of the exact component responsible for reduced postprandial glycemia is difficult to ascertain. However, the insulinotropic properties of milk proteins may be a potential mechanism to explain, at least in part, the inverse relationship between dairy consumption and cardiometabolic diseases [30, 31].

Furthermore, the increased insulin response associated with milk may aid in postprandial vasodilation [34]. Insulin receptors are found on endothelial and vascular smooth muscle cells and binding initiates vasodilation. Postprandial hyperinsulinemia due to dairy proteins may serve to

increase limb blood flow and capillary recruitment for nutrient disposal [35, 36]. This increased clearance of glucose and triglycerides may lead to an attenuated impairment of postprandial vascular dysfunction. However, the precise mechanisms whereby milk and dairy products exert beneficial effects on metabolism and vascular health are unclear. The focus of this review is to evaluate the relationships between metabolic dysfunction, postprandial metabolism, and vascular dysfunction and to discuss the potential role of milk in attenuating these impairments.

PURPOSES AND HYPOTHESES

It is well accepted that the progression of atherosclerosis is primarily a postprandial phenomenon [37]. Indeed, postprandial triglyceride levels are better predictors of atherosclerosis and coronary artery disease than fasting triglycerides [16, 17]. In addition, epidemiological data indicates that postprandial hyperglycemia is associated with increased risk of CVD and mortality [18, 38, 39]. Chronically high consumption of milk and milk products are associated with a reduced risk of diabetes [24-26] and CVD [40] and have an inverse relationship with MetS [27]. A potential mechanism underlying how dairy products may reduce the risk of metabolic dysfunction is thought to be through reductions in postprandial rise in glycemia and triglyceridemia when milk and milk products are consumed [30, 31]. Despite their low glycemic index, milk products are insulinotropic, and the associated postprandial insulinemia may facilitate the clearance and storage of glucose and triglycerides after a meal [29, 41, 42]. However, the complete physiological mechanisms have yet to be described.

Study #1:

The purpose of study #1 was to determine the effect of non-fat milk in attenuating the postprandial hyperglycemic surge and its associated physiological mechanisms. We hypothesized that consuming one serving of non-fat milk with a standard oral glucose tolerance test (OGTT) would attenuate postprandial hyperglycemia. Additionally, we hypothesized that the postulated improvement would be associated with increased postprandial insulin secretion as well as insulin-mediated endothelial vasodilation and whole-limb perfusion.

Study #2:

The purpose of the study #2 was to determine the effect of non-fat milk on attenuating the postprandial surge in plasma triglycerides after a high fat meal and its associated physiological mechanisms. We hypothesized that consuming one serving of non-fat milk with a high fat tolerance test (HFTT) would attenuate postprandial hypertriglyceridemia. Additionally, we hypothesized that the postulated improvement in postprandial metabolic response due to the consumption of non-fat milk would be associated with increased postprandial insulin secretion as well as insulin-mediated endothelial vasodilation and whole-limb perfusion.

NON-FAT MILK ATTENUATES ACUTE HYPERGLYCEMIA AND IMPROVES ENDOTHELIUM-DEPENDENT VASODILATION IN INDIVIDUALS WITH ELEVATED ANDROID BODY FAT

ABSTRACT

Background/Aims: Elevated android body fat increases the risk of developing cardiometabolic diseases. Postprandial hyperglycemia contributes to the pro-atherogenic metabolic state evident in android adiposity. Due to the insulinotropic effect of milk-derived proteins postprandial hyperglycemia has been shown to be reduced with the addition of dairy products. The purpose of this study was to determine if one serving of non-fat milk added to an oral glucose tolerance test (OGTT) could attenuate postprandial hyperglycemia in individuals with elevated android adiposity independent of the effects of the milk's protein content and whether these improvements would be associated with metabolic and/or peripheral hemodynamic effects. **Methods:** In this placebo-controlled, randomized, crossover experimental study, 29 overweight/obese adults (26±1 y) consumed an OGTT beverage (75 g glucose) combined with either non-fat milk (227 g) or a placebo control (12 g lactose + 8 g protein + 207 g water). **Results:** In the whole sample, blood glucose and insulin concentrations increased over time in both trials with no significant differences between trials. Relative increases in peak blood glucose response were significantly related to android body fat ($p<0.05$). When the subjects were divided into tertiles of android body fat, the highest tertile displayed attenuated hyperglycemic responses as well as improvements in flow-mediated dilation during the milk intervention. **Conclusions:** The present findings indicate that a single serving of non-fat milk may attenuate acute hyperglycemia in individuals with elevated android body fat offering a simple and convenient option for managing elevations in blood glucose.

INTRODUCTION

Obesity has long been accepted as a clinical risk factor for cardiovascular and other chronic diseases [43, 44]. In particular, android obesity is associated with greater risk of developing cardiometabolic diseases than other adipose storage patterns [45] as it exacerbates metabolic disturbance [46]. The visceral adipose tissue that coexists with android obesity is associated with insulin resistance as well as reduced glucose disposal and oxidation [47]. Traditionally, fasting plasma glucose has been used as a clinical determinant of CVD risk. However, as individuals spend the majority of their time in a postprandial state, acute responses to plasma glucose following an oral glucose tolerance test (OGTT) are better predictors of relative CVD risk [18]. Indeed, increases in postprandial hyperglycemia following a high carbohydrate meal contribute importantly to a pro-atherogenic metabolic state [21] in part through attenuating endothelium-dependent vasodilation [23, 48].

Chronically high dairy consumption is inversely associated with the risk of metabolic syndrome, type 2 diabetes, and CVD [30, 31]. Importantly, the beneficial effects of dairy on metabolic and clinical parameters are evident in individuals with elevated risk for CVD [27, 49]. However, there appears to be a distinct "floor effect" associated with dairy intake, in which little to no changes were observed in relatively healthy individuals with normal or low baseline levels [50]. The complete mechanism underlying the association between dairy intake and cardiometabolic diseases is not clear. However, dairy products may attenuate the risk of metabolic dysfunction through reductions in postprandial glycemia when milk and milk products are consumed with a meal. Recent research has demonstrated favorable effects in glycemic management when dairy products are combined with high carbohydrate meals [51, 52]. The majority of relevant findings in this area attribute attenuated hyperglycemia to the insulinotropic properties of dairy proteins [53-55]. In fact, milk-derived proteins elicit dose-dependent decreases

in postprandial hyperglycemia when consumed with a high carbohydrate meal [53]. However, no study has yet investigated whether milk can attenuate postprandial hyperglycemia independent of its protein component. Additionally, it is unknown if the beneficial effects of milk are mediated by changes in peripheral vasodilation and hemodynamics.

Therefore, the purpose of this study was to determine whether a serving of non-fat milk could attenuate postprandial hyperglycemia independent of its protein content. The effect of non-fat milk was compared with a placebo drink matched for macronutrient and caloric content in a double-blind fashion. In this study design, any differences in postprandial hyperglycemia between the non-fat milk and placebo trials could not be attributed to the macronutrient, and specifically the protein content, of milk. Because postprandial metabolism is affected greatly by diet and physical activity, these lifestyle factors were tightly controlled. Since android obesity is associated with poorer metabolic health, we sought to determine the influence of android body fat on the milk's ability to reduce postprandial hyperglycemia. Based on the observation that the effects of dairy intake do not appear to manifest in relatively healthy individuals [50], we hypothesized that milk would attenuate an acute hyperglycemic load compared with a macronutrient, caloric control preferentially in individuals with the greatest level of adiposity. Moreover, we posited that these postulated improvements would be associated with peripheral vasodilation.

METHODS

Study Population. A total of 29 adults with a mean (±SEM) age of 26±1 y were studied. Participants were recruited via advertisements and fliers from the local community. Inclusion criteria were as follows: apparently healthy, sedentary (physical activity <3 d/wk), overweight or obese (BMI $\geq$ 25.0 kg/m^2), nonsmokers, no overt signs of chronic diseases on physical examination or medical health history, normal blood chemistry as assessed by fasting glucose and lipid panel,

no cardiovascular-acting medications, and no pregnancy. Participants who were lactating or presented with dairy allergies, lactose intolerance, or alcohol abuse were excluded from the study. Participants were required to maintain their normal routine diet and exercise habits for the duration of the study. After being informed about the study verbally and in writing, all participants gave their informed consent. All procedures were reviewed and approved by the Internal Review Board at The University of Texas at Austin.

Study Design. A placebo-controlled, randomized, crossover experimental design was used for the present investigation. Each participant underwent both non-fat milk and placebo treatments with an oral glucose tolerance test (OGTT). Each treatment was preceded by two consecutive days of diet and physical activity controls. Treatments were separated by a washout period of at least 1 week.

Experimental Protocol. During the screening visit, body composition was assessed using dual-energy X-ray absorptiometry (DXA; see below). This was done to calculate daily caloric requirements for the standardized meals. Standardized meals were matched for energy content (isocaloric; 60% carbohydrate, 15% protein, and 25% fat) and were provided to participants to consume on Days 1-2. The participants were provided with a dietary record log and were instructed to consume the same meals and record the timing of the meals on both days to better replicate the dietary controls prior to the second treatment. Alcohol and caffeine intake were prohibited starting in the evening before Day 1. In addition to the diet controls, the participants were instructed to maintain their normal daily activity but refrain from both formal and recreational exercises. To confirm this, participants were provided with and required to wear pedometers during the waking hours of Days 1-2.

Following these two control days, participants reported to the laboratory on the morning of Day 3 for fasted, resting measures followed by one of the treatments. An intravenous catheter was inserted into the antecubital vein, and fasting blood samples were collected. Upon conclusion of the resting measures, participants consumed a fruit punch flavored, standard OGTT beverage (100 g glucose) combined and mixed with either: 8 oz of non-fat milk (227 g) or 8 oz of the control drink (12 g lactose + 8 g whey protein + 207 g water). The control drink was identical to non-fat milk in macronutrient and calorie contents. Participants were instructed to consume the test beverages within 5 minutes. After ingestion of test solutions, the participants remained in the supine position during the two-hour postprandial period in the quiet, temperature-controlled laboratory.

Measurements. Body composition and android body fat were estimated noninvasively using the total body scan by DXA (GE Lunar, Chicago, IL) [56]. For measuring android fat, a region of interest was automatically defined by the software, in which the caudal limit was placed at the top of the iliac crest and its height set to 20% of the distance from the top of the iliac crest to the base of the skull to define its cephalad limit. During each treatment, blood samples were collected at 10, 20, 30, 45, 60, 90, and 120 minutes during the postprandial period. These samples were later analyzed for plasma glucose, insulin, glucagon, and gastric inhibitor polypeptide (GIP) concentrations. Commercially available assay kits were used to determine plasma concentrations of glucose (Point Scientific, Canton, MI), GIP (RayBio, Norcross, GA) and insulin (Mercodia, Uppsala, Sweden). A commercially available radioimmunoassay kit was used to assess glucagon (EMD Millipore, Darmstadt, Germany) [57]. The net incremental area under the curve (iAUC) for plasma glucose and insulin was calculated using the trapezoidal method [57] as it was shown to be more strongly correlated with glycemic rise than total AUC [58].

Vascular function was measured at baseline, 30 min, and end of the postprandial period (120 min). To assess vascular endothelium-dependent vasodilation, flow-mediated dilation (FMD) was performed as previously described [59]. Briefly, brachial artery diameters and blood flow velocity were measured from images derived from an ultrasound machine (iE33, Philips Medical, Bothel, WA) equipped with a high-resolution linear-array transducer. A longitudinal image of the brachial artery was acquired 5-10 cm proximal to the antecubital fossa. A blood pressure cuff, placed on the forearm 3-5 cm distal to the antecubital fossa, was inflated to 50 mmHg above resting systolic blood pressure or a maximum of 200 mmHg for 5 minutes. After cuff deflation, ultrasound-derived measurements of the brachial artery diameters and blood velocity were taken for 3 minutes. FMD was calculated as a percent increase in brachial artery diameter at the post-blood flow occlusion compared with the pre-blood flow occlusion.

Blood flow and vascular conductance were measured in the common femoral artery using the ultrasound machine (iE33, Philips Medical, Bothel, WA) as previously described [60, 61]. To minimize turbulence from the bifurcation, the measurements were performed below the inguinal ligament, approximately 2-3 cm above its bifurcation into the profundus and superficial branch. Mean blood velocity measurements were performed with the insonation angle <60 degrees. Blood flow was calculated from the following formula: (mean blood velocity) x (circular area) x 6 x 10^4. The data were analyzed by the same investigator, who was blinded to the identity of the participant and the treatment. Femoral artery vascular conductance was calculated as femoral blood flow / mean arterial pressure.

Statistical Analyses. Two-way (treatment x time) ANOVA with repeated measures were used to analyze the effects of treatment solutions. Tukey Least Significant Difference Test (LSD) was used for all post-hoc comparisons. Incremental area under the curve (iAUC) for plasma

glucose, insulin, glucagon, and GIP concentrations were calculated. Linear regression analyses were performed to determine associations between insulin and glucose iAUCs for both trials at 30 and 120 minutes. As well as the relation between relative increases in peak blood glucose concentration [(peak blood glucose – baseline blood glucose)/ baseline blood glucose] and android body fat levels. Changes in FMD were compared between conditions with paired one-tailed t-test. All data were expressed as means±SEM.

RESULTS

Selected participant characteristics are reported in Table 1. Despite being overweight and obese, all participants had optimal or near optimal blood pressure and fasting blood glucose, lipid, and lipoprotein concentrations.

As shown in Figure 1, blood glucose and insulin concentrations increased over time in both the milk and the control drink trials. There were no significant differences in glucose and insulin concentrations or glucose and insulin iAUC between the non-fat milk and control over the two-hour postprandial period. Glucose iAUC was related to insulin iAUC (R^2=0.40, $p<0.05$) for 30 minutes, and the association was stronger when the entire 120 minutes were included (r^2=0.71, $p<0.05$). As depicted in Table 2, there were no significant differences in plasma glucagon or GIP responses between the test beverages over the two-hour postprandial period. Similarly, there was no difference between the test beverages for glucagon or GIP iAUC (data not reported).

Relative increases in peak blood glucose concentration were significantly related to android body fat levels ($r=0.27$, $p<0.05$). In order to determine the influence of abdominal body fatness on metabolic responses, participants were divided into tertiles of android body fat. The participants in the highest tertile of android body fat (>50% android body fat) composed of 10 adults (80%

men and 20% women) displayed attenuated hyperglycemic responses when supplemented with non-fat milk, compared with the control beverage (Figure 2).

Whole group vascular and hemodynamic measures are presented in Table 3. Heart rate, mean blood pressure, and brachial artery FMD did not change significantly during the observation period in both treatments. Femoral artery blood flow and vascular conductance demonstrated time effects with no differences between test beverages. In the sub-group analysis involving the highest tertile of android body fat group, FMD was improved ($p<0.05$) with the milk supplementation (Figure 3).

DISCUSSION

In the entire sample of overweight and obese participants who had otherwise normal metabolic health, there were no differences in metabolic and hemodynamic responses between non-fat milk and the control drink that was matched for macronutrient and total calories. In individuals with the highest android obesity, however, non-fat milk attenuated acute hyperglycemia. The beneficial effects of dairy intake were associated with the elevated endothelium-dependent vasodilation. These findings suggest that milk consumed with a high carbohydrate meal may reduce hyperglycemic responses preferentially in individuals with less favorable cardiometabolic profile and that these metabolic effects may be related to hemodynamic improvements.

Previous studies have reported that when low-fat milk was consumed ad-libitum with a meal, post-meal blood glucose level was decreased [51, 52]. However, because neither the foods nor beverage intakes were controlled, it remains unknown if a single serving of non-fat milk, which is typically consumed with a meal, is sufficient to induce suppression of hyperglycemia after the high carbohydrate intake. In the present study, we controlled and standardized the amount of

carbohydrate meal as well as milk intake. Additionally, because type and amount of prior physical activity and meals could significantly affect postprandial metabolism and vascular responses, we implemented strict dietary and physical activity controls prior to each treatment. We found no significant differences in glycemic responses between the trials involving non-fat milk and those with the control drink that included the same amount of protein. These results confirm previous findings that at least some of the beneficial effects of milk on glycemic responses can be attributed to its protein content [53-55].

In general, studies investigating the effects of specific milk proteins are more conclusive in terms of demonstrating beneficial effects on glycemic responses especially in individuals with elevated risks. For example, the consumption of whey protein with a high glycemic meal decreased postprandial serum glucose in patients with diabetes [54]. We recruited obese yet young, apparently healthy participants that exhibited fairly normal postprandial metabolic and vascular responses. However, by selecting individuals with highly elevated android body fat, we observed a significant effect of milk on attenuating postprandial hyperglycemia. These results are consistent with the previous observation that the beneficial effects of dairy on metabolic and clinical parameters are more likely to be evident in individuals with elevated risk factors for CVD as there appears to be a distinct "basement effect" associated with dairy intake, in which little to no changes have been observed in healthy individuals with normal or low values [27, 49, 50]. Therefore, the observed effects can likely be extended to, and perhaps greater in, older obese adults, type 2 diabetes, and those with CVD. Clearly, further research in these populations is warranted.

The protein component in non-fat milk is ~20% whey and ~80% casein protein. Although the total protein content of the control drink was closely matched with non-fat milk, it contained only whey protein. Whey protein is more quickly digested and absorbed than casein protein

causing it to appear in the blood stream sooner. Whey protein ingestion reduces postprandial hyperglycemia without increases in C-peptide release or insulin concentrations suggesting that whey may affect glucose clearance by stimulating insulin-independent mechanisms [53, 62]. In spite of this, we observed improvements in the early phase of the OGTT in the highest tertile of android obesity. Therefore, any insulin-independent effects of whey do not appear to be responsible for the observed improvements in glycemic responses. To the best of our knowledge, this is the first study to demonstrate that milk can elicit glycemic improvements independent of the macronutrient composition, more specifically protein content. Importantly, previous studies investigating the effects of milk and milk protein on postprandial hyperglycemia employed less carbohydrate and more protein [53, 54], allowing for a greater potential of protein to limit increases in blood glucose. The present findings indicate that a single serving of milk was effective in attenuating the glycemic effect of high carbohydrate meals at least in individuals with highly elevated android obesity.

Other components of milk may be responsible for the attenuated glycemic response. The combined vitamin and mineral content comprises less than 1% of milk, but this rather small volume could offer significant functional properties [63]. Oral magnesium supplementation, for example, improves insulin sensitivity, glucose homeostasis, and HbA1c levels in diabetic patients [64]. Several intervention studies have shown improved glycemic responses with vitamin D treatment, but these effects may be specific to individuals who were deficient at baseline or those who had preexisting metabolic disorders [63, 65]. The micronutrient profile of dairy could contribute to attenuated hyperglycemia as the cellular influx of calcium plays a pivotal role in nutrient intake and in endothelial-dependent vasodilation, and magnesium is essential for optimal coupling and signaling through the insulin receptor [63, 66].

Nearly all studies showing the beneficial effects of dairy intake have attributed the favorable metabolic effects to the insulinotropic properties of milk proteins. Indeed, compared with other protein sources, milk demonstrates a larger insulin response up to 240 min post-meal [30]. By controlling for the amount of dairy protein in the test beverages, we ensured that any positive changes were independent of the effects of milk proteins on insulin secretion. As there were no differences in insulin responses between beverages, the effects of dairy on acute hyperglycemia in this study appear to be insulin-independent.

Our integrative physiological approach allowed us to gain insight into potential mechanisms underlying the effects of milk on postprandial metabolism. Specifically, we addressed the hypothesis that improvements in postprandial hyperglycemia would be associated with increases in peripheral perfusion and vasodilation. Compared with an isocaloric volume of rice milk, obese individuals demonstrate improved endothelial-dependent vasodilation with low-fat milk [67]. However, neither a meal nor the carbohydrate or protein differences were controlled between the test beverages. Additionally, whey-derived protein elicited improved endothelium-dependent vasodilation at 120 minutes in mildly hypertensive, overweight participants [68]. We also observed the beneficial effect of milk intake on FMD at the end of the OGTT although blood glucose concentration was no longer different between the trials. One may argue that the reduced glycemic load at 30 minutes may have elicited a delayed effect in improved vasodilation that became evident at 120 minutes. Nevertheless, the improvement in FMD implies vascular protective effects of milk and significant therapeutic applications for at-risk individuals.

Importantly, the present findings are the first to demonstrate that non-fat milk is capable of attenuating postprandial hyperglycemia independent of the hypoglycemic effects of dairy protein in individuals with elevated android obesity. Based on the previous epidemiological studies,

attenuating postprandial hyperglycemia offers tremendous potential to reduce future CV risks. The present findings indicate that a single serving of non-fat milk was sufficient to attenuate acute hyperglycemia at least in individuals with highly elevated android obesity. This offers a simple, convenient, and easily implemented option for managing elevations in blood glucose in individuals at high risk for developing CVD.

ACKNOWLEDGEMENTS

This work was supported by a research grant (#1198) from the Dairy Research Institute (HT). The funding source had no involvement in study design; in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the article for publication. This trial was registered at clinicaltrials.gov as NCT 02894112.

Table 1. Selected Participant Characteristics.

Variable	Mean ± SEM
Age (yr)	26 ± 1
Men/Women (n)	17/12
Height (cm)	172 ± 1
Body Weight (kg)	92.7 ± 2.0
BMI (kg/m^2)	31.6 ± 0.9
Total Body Fat (%)	39 ± 2
Android Body Fat (%)	48 ± 2
Systolic BP (mmHg)	116 ± 2
Diastolic BP (mmHg)	77 ± 2
Total Cholesterol (mg/dl)	180 ± 8
LDL Cholesterol (mg/dl)	108 ± 7
HDL Cholesterol (mg/dl)	48 ± 7
Triglycerides (mg/dl)	107 ± 17
Blood Glucose (mg/dl)	92 ± 2

(n=29); BMI = body mass index, BP = blood pressure, LDL = low density lipoprotein, HDL = high density lipoprotein

Table 2. Changes in metabolic hormone concentrations throughout the oral glucose tolerance test (OGTT) with non-fat milk or the control drink.

Measure	Trial	Oral Glucose Tolerance Test							
		0	**10**	**20**	**30**	**45**	**60**	**90**	**120**
Glucagon (pg/mL)	Milk	106 ± 5	106 ± 5	100 ± 5*†	93 ± 5*†‡	91 ± 6*†‡	91 ± 5*†‡	87 ± 5*†‡§	86 ± 5*†‡§
	Control	107 ± 5	109 ± 5	104 ± 5†	93 ± 4*†‡	91 ± 4*†‡	89 ± 4*†‡	82 ± 5*†‡§¶#	83 ± 4*†‡§¶#
GIP (pmol/L)	Milk	18 ± 3	———	———	19 ± 3	———	18 ± 2	———	———
	Control	19 ± 3	———	———	18 ± 3	———	17 ± 3	———	———

GIP = gastric inhibitory peptide, * compared with baseline, † Compared with 10 minutes, ‡ compared with 20 minutes, § compared with 30 minutes, ¶ compared with 45 minutes, # compared with 60 minutes; ($p<0.05$)

Table 3. Changes in vascular and hemodynamic measures at baseline and at the end of the oral glucose tolerance test (OGTT) with non-fat milk or the control drink.

Measure	Trial	OGTT		
		Baseline	**30 min**	**120 min**
Mean Blood Pressure (mmHg)	Milk	84 ± 3	81 ± 2	79 ± 3
	Control	85 ± 2	83 ± 1	77 ± 4
Heart Rate (bpm)	Milk	72 ± 3	70 ± 2	71 ± 2
	Control	68 ± 2	71 ± 2	71 ± 2
Brachial Flow-Mediated Dilation (%)	Milk	7.6 ± 0.6	8.4 ± 0.6	8.4 ± 0.6
	Control	8.7 ± 0.6	7.1 ± 0.6	8.0 ± 0.7
Femoral Blood Flow (ml/min)	Milk	482 ± 42	313 ± 28*	413 ± 45†
	Control	463 ± 52	310 ± 31*	353 ± 27*
Femoral Vascular Conductance (AU)	Milk	5.9 ± 0.6	3.8 ± 0.4*	4.9 ± 0.5
	Control	5.6 ± 0.7	3.7 ± 0.4*	4.4 ± 0.4

* compared with baseline, † Compared with 30 minutes

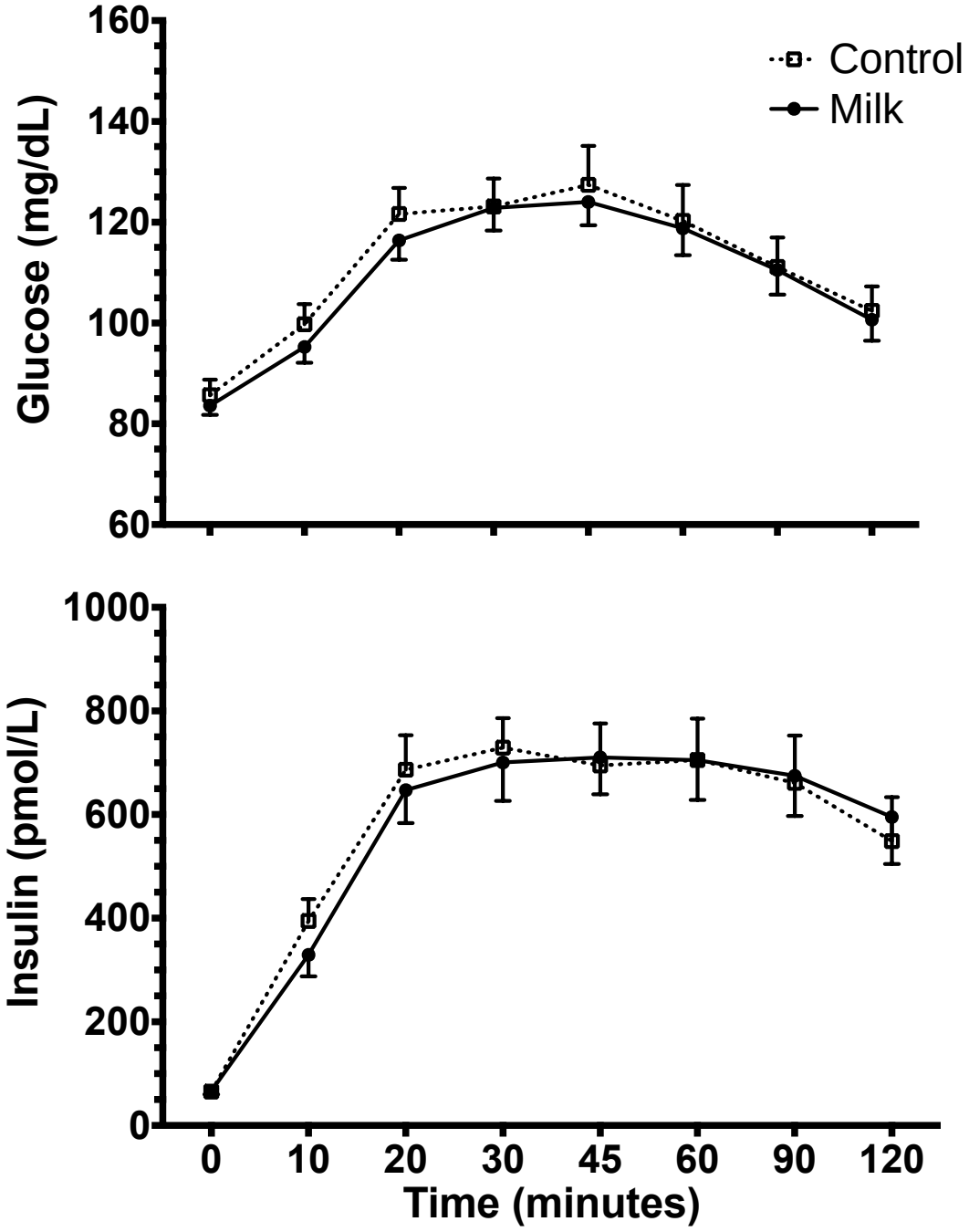

Figure 1. Whole group glucose and insulin response.

Changes in blood glucose and insulin concentrations during the OGTT with non-fat milk or the control drink (n=29). No treatment by time effect ($p>0.05$). Values are mean ± SEM.

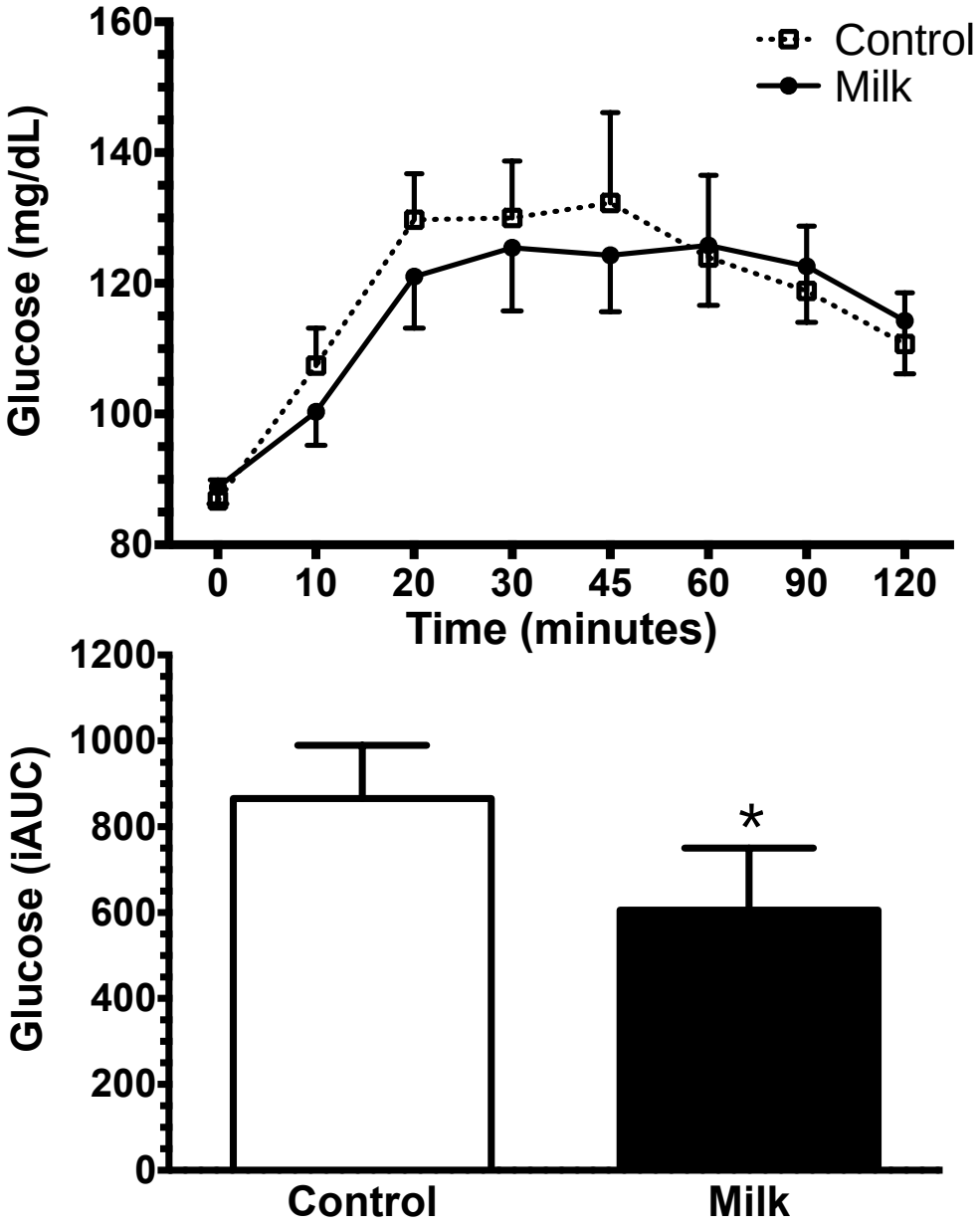

Figure 2. Subgroup blood glucose response.

Changes in blood glucose concentration and mean glucose integrated area under the curve (iAUC) at 30 minutes during the OGTT with non-fat milk or the control drink in the participants in the highest android body fat tertile (n=10). Values are mean ± SEM.

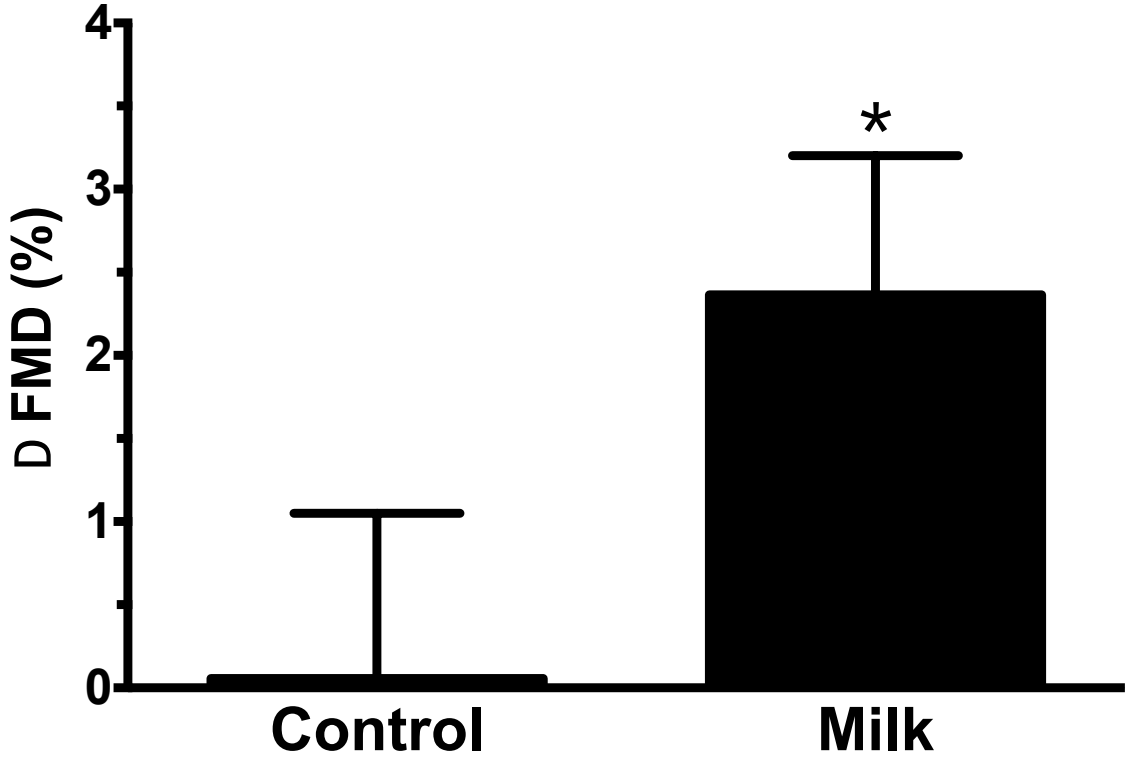

Figure 3. Subgroup flow-mediated dilation.

Change in flow-mediated dilation (FMD) from baseline to the end of the OGTT with non-fat milk or the control drink in the participants in the highest android body fat tertile (n=10). Values are mean ± SEM.

NON-FAT MILK IMPROVES ACUTE HYPERTRIGLYCERIDEMIA IN INDIVIDUALS WITH CHRONIC CONSUMPTION OF HIGH FAT DIET

ABSTRACT

Background/Aims: Repeated exposure to elevated postprandial triglycerides, as seen with typical Western diets, contributes to atherosclerosis and vascular disease. The purpose of this study was to determine if a single serving of non-fat milk added to a high fat tolerance test could attenuate postprandial hypertriglyceridemia in individuals who consume a high fat diet. **Methods:** In this placebo-controlled, randomized, crossover experimental study, 30 overweight/obese adults consumed a high fat tolerance test meal combined with either non-fat milk, carbohydrate control (CHO), or caloric control (CAL). **Results:** In the whole sample, plasma triglycerides increased over time with no significant differences between interventions. Peak plasma triglyceride levels were significantly related to dietary fat intake ($r=0.30$, $p<0.05$). When participants were ranked and divided into tertiles based on habitual dietary fat intake, the high fat diet group exhibited reduced triglyceride net integrated area under the curve (iAUC) when supplemented with non-fat milk. No significant differences in hemodynamic measures (brachial flow-mediated dilation and femoral vascular conductance) were observed between the milk and caloric control trials for either the low fat or high fat diet groups. **Conclusions:** A single serving of non-fat milk may attenuate acute hypertriglyceridemia in individuals who chronically consume a high fat diet, offering a simple and easily implemented option for managing elevations in postprandial triglycerides.

INTRODUCTION

Atherosclerosis is responsible for over 40% of deaths in the U.S. [69]. Traditionally, fasting plasma triglyceride has been used as an important risk factor for atherosclerosis. However, as most individuals spend the majority of their awakening time in a postprandial state, acute responses in plasma triglycerides following a meal have been shown to be better predictors of relative cardiovascular disease (CVD) risk [14, 15]. Indeed, postprandial lipemia is associated with reduced vascular reactivity and impaired endothelial function [22, 70, 71]. The magnitude of increase in postprandial plasma triglycerides is directly proportional to the fat content in meals [20, 72, 73]. As such, multiple high fat meals throughout the day exemplified by typical Western diets results in the prolonged presence of elevated plasma triglycerides. Repeatedly exposing the vasculature to hypertriglyceridemia causes endothelial dysfunction, which may increase the risk of developing atherosclerosis.

Epidemiological studies suggest that chronically high consumption of milk and dairy products are inversely associated with the overall risk of atherosclerosis [27, 28, 49, 74, 75]. A potential mechanism underlying how dairy products reduce the risk of atherosclerosis may be through the attenuation in the postprandial rise in triglyceridemia when milk and milk products are consumed with a high fat meal [30, 33, 76, 77]. However, the available evidence is inconclusive and highly controversial. For example, the postprandial appearance of triglycerides was decreased with the consumption of large doses of milk-derived proteins [77]. In contrast, no difference was found in postprandial triglycerides between milk, a carbohydrate control, and a caloric control [33].

Milk proteins demonstrate an insulinotropic effect upon absorption in the gut that may induce the rapid release of insulinotropic amino acids and gastric inhibitor polypeptide (GIP), which in turn augments insulin secretion [29]. As insulin-mediated vasodilation is achieved

through a nitric oxide dependent mechanism, postprandial hyperinsulinemia and the resultant vasodilation could be a mechanism underlying the potential effect of milk protein on postprandial hypertriglyceridemia [35]. However, hemodynamic studies have not been performed in conjunction with the effects of milk and milk proteins to confirm this possibility.

Thus, the main aim of the present study was to determine if a single serving of non-fat milk could attenuate postprandial hypertriglyceridemia induced by a high fat tolerance test in individuals who chronically consume a high fat diet. Overweight and obese individuals who consume high fat diets are at an increased risk of developing atherosclerosis [78] and often present with sub-optimal metabolic profiles. These individuals may be the biggest beneficiaries of the proposed dietary regimen. Accordingly, participants were ranked and divided into tertiles based on habitual dietary fat intake. As secondary outcomes, we also evaluated whether these improvements would be associated with hyperinsulinemic and/or vasodilatory effects, including endothelial-dependent vasodilation and whole limb perfusion. Employing a carbohydrate control and a macronutrient/caloric control allowed us to determine whether milk, independent of its protein content, was capable of attenuating postprandial triglyceride response.

METHODS

Participants. A total of 30 young adults with a mean (±SEM) age of 26±1 y were studied. They were recruited via advertisements and fliers from the local community. Inclusion criteria were as follows: healthy, sedentary (physical activity <3 d/wk), overweight or obese (BMI ≥ 25.0 kg/m^2), nonsmoking, no overt signs of chronic diseases as assessed by physical examination and/or medical health history, normal blood chemistry as assessed by fasting glucose and lipid panel, no cardiovascular-acting medications, and no pregnancy. Participants who were lactating or reported dairy allergies, lactose intolerance, and/or alcohol abuse were excluded from the study.

Participants were required to maintain their normal routine diet and exercise habits for the duration of the study participation. After being informed about the study verbally and in writing, all participants gave their informed consent. All procedures were reviewed and approved by the Internal Review Board.

Study Design. A placebo-controlled, randomized, crossover experimental design was used for the present dietary intervention trials. Each participant underwent the following treatments: non-fat milk, a carbohydrate control, and a total caloric control. Each treatment was preceded by two consecutive days of strict dietary and physical activity controls. Treatment interventions were randomized and separated by a washout period of at least 1 week.

Experimental Protocol. Dietary intake was reported with a 3-day, self-report food log and analyzed by a registered dietician with Nutrition Pro Software (Axxya Systems, Woodinville, WA). During the screening visit, body composition was estimated noninvasively using the total body scan by dual energy x-ray absorptiometry (GE Lunar, Chicago, IL) [56]. This was done prior to the study to calculate daily caloric requirements for the standardized meals given prior to the main protocol. Standardized meals were matched for energy/calorie content (isocaloric; 60% carbohydrate, 15% protein, and 25% fat) and were provided to participants to consume on Days 1-2. The participants were provided with a dietary record log and instructed to consume the same meals and record the timing of the meals on both days to better replicate the dietary controls prior to the other treatments. Alcohol and caffeine intake were prohibited starting in the evening before Day 1. In addition to the dietary controls, the participants were instructed to maintain their normal physical activity, but refrain from both formal and recreational exercises. To confirm this, participants were provided with and required to wear pedometers during the waking hours of Days 1-2.

Following these two control days, participants reported to the laboratory on the morning of Day 3. Following the resting measurements of vascular functions, an intravenous catheter was inserted into the antecubital vein, and fasting blood samples were collected. Upon conclusion of these resting measures, participants consumed a high fat meal consisted of corn chips (H.E.B., San Antonio, TX), which were 68% fat/serving. To normalize the high fat load, participants were given 1 g of dietary fat/kg of body weight. This high fat meal was provided with 8 oz of non-fat milk (227 g liquid weight), 8 oz of carbohydrate (CHO) control drink (12 g lactose + 215 g water), or 8 oz of the caloric (CAL) control drink (12 g lactose + 8 g whey protein + 207 g water). The carbohydrate control drink was identical to non-fat milk in carbohydrate content, and the caloric control drink was identical to non-fat milk in macronutrient and calorie contents. Participants were instructed to consume the test meal and beverage within 20 minutes. After ingestion of test meal and beverages, the participants remained sedentary in the quiet, temperature-controlled laboratory during the 4-hour postprandial period.

Measurements. During each treatment, blood samples were collected at baseline, 30, 60, 90, 120, 180, and 240 minutes during the postprandial period. These samples were later analyzed for plasma glucose, insulin, glucagon, glucagon-like peptide-1 (GLP-1), and gastric inhibitor polypeptide (GIP) concentrations. Due to financial constraint, GLP-1 and GIP concentrations were analyzed only for baseline, 30, 60, and 120 minutes. These time points were selected because significant changes in these hormones were expected to occur early in the postprandial phase. Commercially available assay kits were used to determine triglyceride and glucose concentrations (Point Scientific, Canton, MI). Enzyme-linked immunosorbent assays were used to assess plasma concentrations of GIP and GLP-1 (RayBiotech, Inc., Norcross, GA) and insulin (Mercodia, Uppsala, Sweden). Glucagon concentrations were determined by radioimmunoassay (EMD

Millipore, Darmstadt, Germany) [57]. The net incremental area under the curve (iAUC) for plasma triglycerides was calculated using the trapezoidal method [57].

Vascular function was measured at baseline, midway (120 min), and end of the postprandial period (240 min). As an index of vascular endothelium-dependent vasodilation, flow-mediated dilation (FMD) was measured as previously described [59]. Briefly, brachial artery diameter and blood flow velocity were assessed from images derived from an ultrasound machine (iE33, Philips Medical, Bothel, WA) equipped with a high-resolution linear-array transducer. A longitudinal image of the brachial artery was acquired 5-10 cm proximal to the antecubital fossa. A blood pressure cuff, placed on the forearm 3-5 cm distal to the antecubital fossa, was inflated to 50 mmHg above resting systolic blood pressure or a maximum of 200 mmHg for 5 minutes. After cuff deflation, ultrasound-derived measurements of the brachial artery diameters and blood velocity were taken for 3 minutes. FMD was calculated as a percent increase in brachial artery diameter at the post-blood flow occlusion compared with the pre-blood flow occlusion.

Blood flow and vascular conductance were measured in the common femoral artery using the ultrasound machine (iE33, Philips Medical, Bothel, WA) as previously described [60, 61]. To minimize turbulence from the bifurcation, the measurements were performed below the inguinal ligament, approximately 2-3 cm above its bifurcation into the profundus and superficial branch. Mean blood velocity measurements were performed with the lowest possible insonation angle that was always <60 degrees. Blood flow was calculated using the following formula: (mean blood velocity) x (circular area) x 6 x 10^4. The data were analyzed by the same investigator, who was blinded to the identity of the participant and the treatment. Femoral artery vascular conductance was calculated as femoral blood flow / mean arterial pressure.

Statistical Analyses. All data were expressed as mean±SEM. Two-way (treatment x time) ANOVA with repeated measures were used to analyze the effects of treatments on vascular, hemodynamic, and metabolic measures. Tukey's Least Significant Difference (LSD) test was used for all post-hoc comparisons. Participants were ranked based on dietary fat intake from the normal diet as self-reported in the 3-day food log and divided into tertiles for analyses. Differences in triglyceride iAUC were compared between subgroups with paired two-tailed t-tests. Linear regression and correlation analyses were performed to determine the relationship between metabolic measures.

RESULTS

Selected participant characteristics are reported in Table 1. Average BMI of the participants was in the obese category. Despite being obese or overweight, all participants had normal blood pressure and fasting blood glucose, lipid, and lipoprotein concentrations.

During the high fat tolerance test (HFTT), plasma triglyceride concentrations increased over time in all trials with no significant differences between milk and control treatments at any time point (Figure 1A). Moreover, there were no significant differences in triglyceride iAUC between the test beverages over the four-hour postprandial period although there was a trend for milk to reduce triglyceride iAUC (Figure 1B).

Plasma glucose and hormone concentrations are presented in Table 2. Plasma glucose and insulin peaked at 30-60 minutes exhibiting significant time effects for all treatments with no differences between tests. GIP concentration increased ($P<0.05$) for all treatments, whereas GLP concentration did not change significantly for any treatment.

Vascular and hemodynamic measures are presented in Table 3. Mean arterial pressure, heart rate, and brachial FMD did not change during the HFTT in any trials. Vascular conductance and femoral blood flow decreased significantly only in the milk and carbohydrate control trials.

When correlational analyses were performed, peak plasma triglyceride levels during the HFTT were significantly related to dietary fat intake ($r=0.30$, $p<0.05$). The participants were ranked and divided into tertiles of dietary fat intake based on the self-reported 3-day food logs. The low fat diet group (40% men and 60% women) consumed 16% protein, 50% carbohydrate, and 34% fat in their regular diets. The high fat group (70% men and 30% women) consumed 17% protein, 39% carbohydrate, and 44% fat. There were no significant differences in gender/sex, body weight, or BMI between the groups. As shown in Figure 2, compared with the caloric control beverage, the high fat diet group exhibited reduced triglyceride responses when supplemented with milk. No such trend was observed in the low fat diet group. When hypotriglyceridemic effects of the milk were calculated by subtracting the effects from calorie control, such effects were significant only in the high fat diet group. No differences were observed in insulin concentrations or iAUC between the milk and caloric control trials for either the low fat or high fat diet groups. Similarly, for vascular and hemodynamic measures, no differences were observed between the milk and caloric control trials for either the low fat or high fat diet groups.

DISCUSSION

In the entire sample of overweight and obese participants who had otherwise normal metabolic health, there were no differences in metabolic and hemodynamic responses between non-fat milk and the control drinks. However, when participants were divided into tertiles of dietary fat intake, non-fat milk attenuated acute hypertriglyceridemia in individuals who regularly

consumed a high fat diet. These findings suggest that non-fat milk consumed with a high fat meal reduces the hypertriglyceridemic response in individuals who regularly consume a high fat diet.

Our whole group findings that milk does not reduce postprandial triglyceride response compared with carbohydrate or macronutrient/caloric controls are in agreement with previous findings [33]. However, in analyzing the whole group data, we identified dietary fat intake to be a dominant factor predicting postprandial triglyceride response as evidenced by a highly significant association between the two. We then divided participants into tertiles of dietary fat intakes and demonstrated that in those individuals who habitually consumed a high fat diet, a high fat tolerance meal with a macronutrient/caloric control beverage elicited a larger increase in postprandial triglycerides compared with the low fat diet group. However, the larger postprandial triglyceride response was attenuated with the non-fat milk treatment, suggesting that non-fat milk does offer a hypotriglyceridemic effect in those individuals who habitually consume typical Western diets.

The lipid lowering effects seen in the present study may be attributed to dairy's insulinotropic properties. Compared with other protein sources, milk demonstrates a larger insulin response up to 240 min post-meal [30]. Increased insulin concentration during postprandial lipemia limits the hydrolysis and release of stored triglycerides by inhibiting hormone sensitive lipase. Insulin also stimulates lipoprotein lipase causing the hydrolysis and release of triglycerides from lipoproteins for storage [79]. However, there were no differences in insulin response between trials suggesting that insulin may not be the mediating factor eliciting the hypotriglyceridemic response of the non-fat milk intervention in the present study.

In the sub-group analyses using tertiles, the macronutrient/caloric control beverage used in this study was not as effective as non-fat milk in eliciting reductions in postprandial triglycerides in spite of the similar protein content. One potential explanation is the differences in protein

constituents between the two beverages. While the total protein content was matched, non-fat milk consists of 80% casein whereas the macronutrient/caloric control was 100% whey. Casein is a slowly digested protein compared with whey and remains in the stomach longer. During a high energy, mixed meal, the larger casein content in the milk trial likely delayed gastric emptying and reduced the absorption of dietary fat [80]. Therefore, the reduced triglyceride response during the non-fat milk trial may be mediated by a higher casein content compared with the macronutrient/caloric control trial.

Additionally, the micronutrient profile present in the non-fat milk intervention may have contributed to the reduction in postprandial triglycerides. A recent review reported that the inverse relationship between serum vitamin D status and metabolic syndrome may be mediated by reduced hyperlipidemia [81]. Additionally, it has been proposed that the high calcium content in dairy benefits the serum lipid profile. The proposed physiological mechanism is that calcium binding to saturated fatty acids within the intestines forms insoluble soaps that are excreted in the feces. Healthy men consuming milk or yogurt daily containing calcium demonstrated increased fecal fatty acid and bile acid excretion and lower fat absorption during dairy intake [82]. And finally, magnesium has been shown to reduce serum triglycerides, in patients with ischemic heart disease [83], presumably through modifications of several enzymes intricately linked with lipid metabolism [84].

Chronically high dairy consumption is inversely associated with the risk of metabolic syndrome, type 2 diabetes, and CVD [30, 31]. The beneficial effects of dairy intake on metabolic and clinical parameters are evident in individuals with elevated risk for CVD [27, 49], but minimal or negligible in relatively healthy individuals [50]. This “baseline effect” may explain our present finding that the beneficial postprandial effects of milk were not evident in the low fat intake group

because, despite being overweight/obese, the lower dietary fat intake maintained a more optimal metabolic profile, as evident by lower fasting triglycerides.

There are very few previous studies that have examined the effect of complete dairy products on postprandial triglycerides. Most of these studies suffer from a lack of proper controls prior to and/or during the interventions [32, 33]. For instance, a study comparing the effects of a high fat dairy meal, a high fat non-dairy meal served with whole-fat milk, and a high fat non-dairy control meal, found neither dairy meal exhibited different postprandial triglyceride response compared with the control meal [32]. Another study that investigated the effects of non-fat milk against control beverages found serum triglyceride concentrations during the postprandial period did not differ between test meals [33]. However, vascular response as a potential mechanism in the postprandial state was not examined nor did they employ rigorous diet and exercise controls. Therefore, the present study is unique in that it employed proper dietary, as well as physical activity, controls both prior to and during the intervention. Additionally, in attempts to offer underlying mechanisms, ours is the first study to examine the postprandial vascular response to a high fat meal with non-fat milk.

Our integrative physiological approach allowed us to gain insight into the potential mechanisms underlying the effects of dairy products on postprandial metabolism. While we did confirm our hypothesis that consuming one serving of non-fat milk with a high fat tolerance test (HFTT) would attenuate postprandial hypertriglyceridemia in individuals with a chronic high fat diet, we were unable to confirm that the improvement was associated with insulinotropic effects and the subsequent improvements in endothelial function and perfusion. In a previous study, reductions in postprandial FMD caused by hypertriglyceridemia were attenuated with large doses

(50g) of protein intake [85]. Perhaps the smaller protein content used in our trials (8g) was an insufficient stimulus for eliciting improvements in triglyceride-mediated endothelial dysfunction.

One potential limitation of the present study was the use of strict control days. Diet and physical activity were carefully controlled prior to testing as they can modulate postprandial metabolism. However, one may argue that if participants had been allowed to consume their typical diet, an even greater benefit of milk might have been expected. Another potential limitation is that the serving size of fat consumed was relative to body weight meaning each participant consumed a different amount of fat. Finally, the fat content in corn chips is more than 85% unsaturated fat. Therefore, we cannot extend these findings to meals high in the more metabolically harmful saturated fat.

Clearly, the metabolic repercussions of repeated hyperlipidemic exposure elicits greater surges in postprandial triglycerides compared with a low fat diet. Importantly, the present findings are the first to confirm that non-fat milk is capable of attenuating postprandial hypertriglyceridemia in individuals who consume a high fat diet. Based on previous epidemiological studies, attenuating postprandial hypertriglyceridemia offers tremendous potential to reduce future CV risks. A single serving of non-fat milk with a typical Western diet offers a simple, convenient, and easily implemented option for managing elevations in postprandial triglycerides in individuals at risk for developing CVD.

ACKNOWLEDGEMENTS

This work was supported by a research grant (#1198) from the Dairy Research Institute (HT). The funding source had no involvement in study design; in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the article for publication. This trial was registered at clinicaltrials.gov as NCT 02894112.

Table 4. Selected Participant Characteristics.

Variable	Mean ± SEM
Men/Women (n)	18/12
Age (yr)	26 ± 1
Height (cm)	172 ± 1
Body Weight (kg)	93.6 ± 2.4
BMI (kg/m^2)	31.5 ± 0.8
Total Body Fat (%)	39 ± 2
Systolic BP (mmHg)	115 ± 2
Diastolic BP (mmHg)	77 ± 2
Total Cholesterol (mg/dl)	178 ± 7
LDL Cholesterol (mg/dl)	108 ± 7
HDL Cholesterol (mg/dl)	50 ± 3
Triglycerides (mg/dl)	107 ± 16
Blood Glucose (mg/dl)	93 ± 2

(n=30); BMI = body mass index, BP = blood pressure, LDL = low density lipoprotein, HDL = high density lipoprotein

Table 5. Changes in hemodynamic measures during the high fat tolerance test with carbohydrate control drink (CHO), caloric control drink (CAL), or non-fat milk (MILK).

Measure	**Trial**	**High Fat Tolerance Test**		
		Baseline	120 min	240 min
Mean BP (mmHg)	CHO	83 ± 2	83 ± 2	85 ± 2
	CAL	84 ± 2	83 ± 2	83 ± 2
	MILK	83 ± 1	82 ± 2	82 ± 2
Heart Rate (bpm)	CHO	72 ± 2	74 ± 2	73 ± 2
	CAL	71 ± 2	74 ± 2	74 ± 2
	MILK	71 ± 2	72 ± 2	71 ± 2
Brachial Flow-Mediated Dilation (%)	CHO	7.8 ± 0.6	7.9 ± 0.7	9.1 ± 0.8
	CAL	9.3 ± 0.8	8.3 ± 0.7	9.0 ± 0.9
	MILK	9.0 ± 0.8	8.3 ± 0.8	7.9 ± 0.7
Femoral Blood Flow (ml/min)	CHO	476 ± 39	318 ± 26*	359 ± 49*
	CAL	430 ± 33	400 ± 42	405 ± 45
	MILK	465 ± 39	429 ± 63	321 ± 38*†
Femoral Vascular Conductance (AU)	CHO	5.8 ± 0.5	3.9 ± 0.3*	4.3 ± 0.6*
	CAL	5.2 ± 0.4	5.2 ± 0.6	4.9 ± 0.6
	MILK	5.7 ± 0.5	5.6 ± 0.8	4.1 ± 0.5*†

BP = blood pressure, * vs. baseline, † vs. 120 mi

Table 6. Changes in circulating metabolic measures throughout the high fat tolerance test with carbohydrate control (CHO), caloric control (CAL), or non-fat milk.

Measure	Trial	High Fat Tolerance Test						
		Baseline	30 min	60 min	90 min	120 min	180 min	240 min
Glucose (mg/dL)	CHO	97 ± 4	124 ± 5*	113 ± 6*	103 ± 5†	96 ± 5†‡	94 ± 4†‡	93 ± 4†‡§¶
	CAL	95 ± 2	119 ± 5*	116 ± 5*	114 ± 5*	108 ± 6	102 ± 5†‡	94 ± 4†‡
	Milk	93 ± 3	121 ± 5*	112 ± 4*	113 ± 4*	101 ± 4†	95 ± 4†‡§	97 ± 3†‡§
Insulin (pmol/L)	CHO	56 ± 5	465 ± 50*	472 ± 58*	334 ± 55*†‡	280 ± 45*†‡	177 ± 25*†‡§¶	184 ± 24*†‡§¶
	CAL	60 ± 9	469 ± 51*	382 ± 38*†	395 ± 49*	359 ± 45*†§	236 ± 45*†‡§¶	171 ± 31*†‡§¶
	Milk	64 ± 9	525 ± 43*	408 ± 40*†	417 ± 46*†	290 ± 37*†‡§	200 ± 29*†‡§¶	183 ± 30*†‡§¶
GIP (pmol/L)	CHO	26 ± 4	31 ± 4	34 ± 4*	_____	37 ± 5*†	_____	_____
	CAL	32 ± 7	35 ± 5*	32 ± 5	_____	33 ± 5*	_____	_____
	Milk	28 ± 5	32 ± 5	35 ± 6*	_____	35 ± 6*	_____	_____
GLP-1 (pmol/L)	CHO	38 ± 7	36 ± 7	39 ± 8	_____	37 ± 7	_____	_____
	CAL	38 ± 6	36 ± 7	34 ± 7	_____	35 ± 6	_____	_____
	Milk	38 ± 7	35 ± 7	36 ± 7	_____	34 ± 6	_____	_____

GIP = gastric inhibitory peptide, GLP-1 = glucagon like peptide-1, * vs. baseline, † vs. 30 min, ‡ vs. 60 min, § vs. 90 min, ¶ vs. 120 min

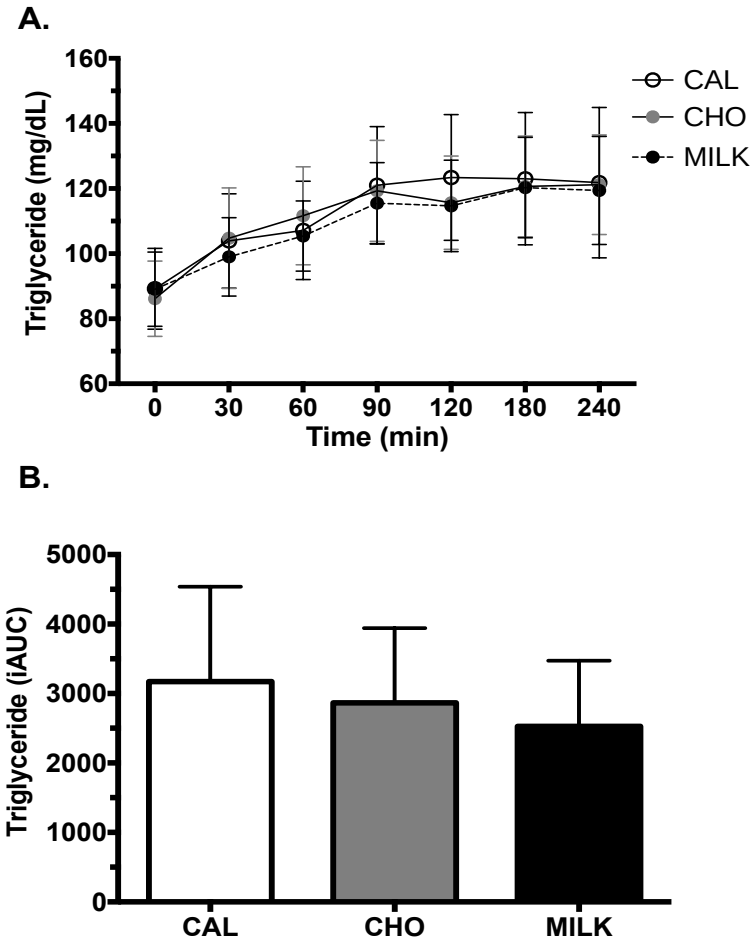

Figure 4. Whole group triglyceride response

A. Changes in plasma triglyceride concentrations during the high fat tolerance test (HFTT) with carbohydrate control drink (CHO), caloric control drink (CAL), and non-fat milk (MILK) (n=30). **B.** Changes in plasma triglyceride integrated area under the curve (iAUC) at 240 minutes during the HFTT with control drinks and non-fat milk. Values are mean ± SEM.

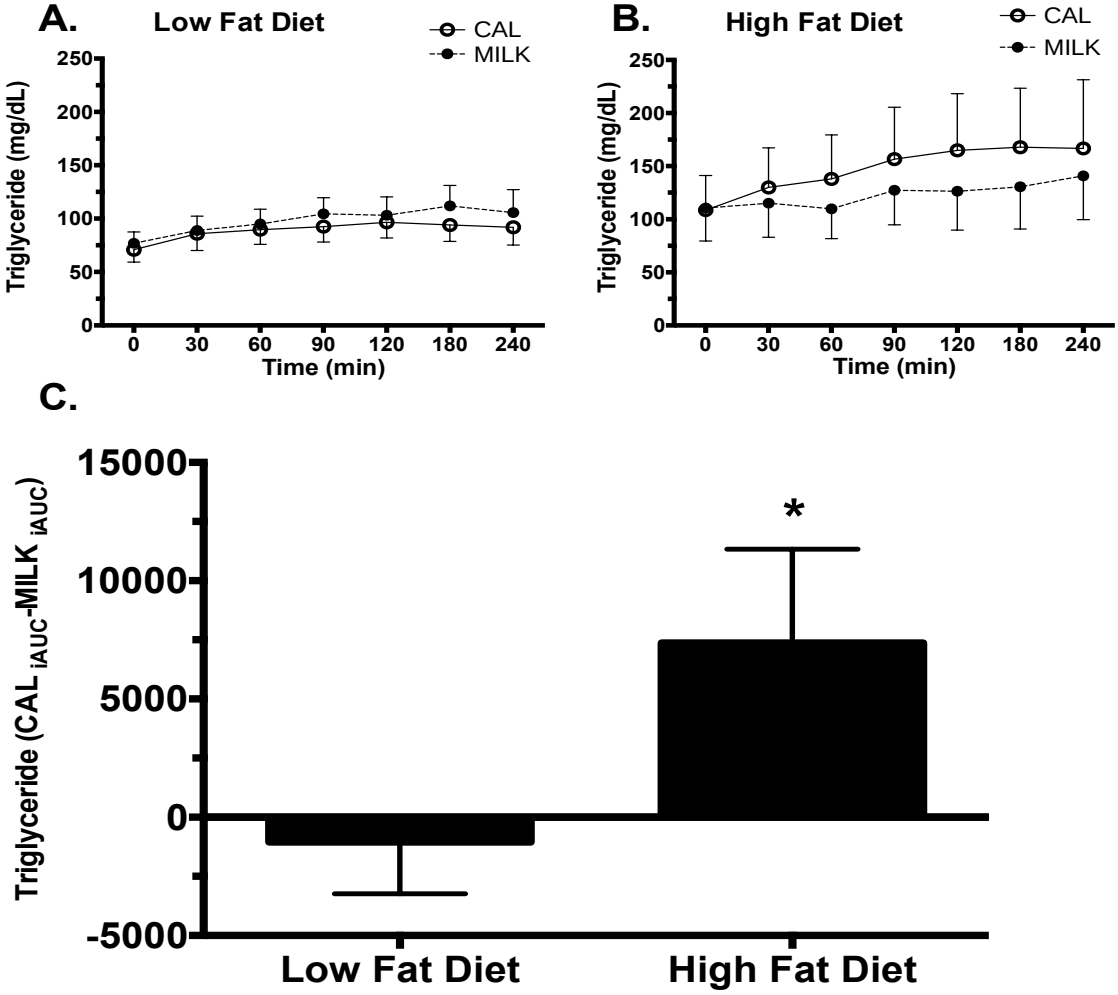

Figure 5. Subgroup triglyceride response.

A. Changes in plasma triglyceride concentrations during the high fat tolerance test (HFTT) with caloric control drink (CAL) and non-fat milk (MILK) in the low fat diet group (n=10) and **B.** high fat diet group (n=10). **C.** Changes in triglyceride integrated area under the curve (iAUC) between trials (CAL iAUC – MILK iAUC). Values are mean ± SEM.

REVIEW OF LITERATURE

METABOLIC SYNDROME

Metabolic Syndrome (MetS) is a constellation of interrelated metabolic risk factors that promote atherosclerotic cardiovascular disease and type 2 diabetes mellitus [1, 2, 86, 87]. Underlying risk factors such as age, body weight, smoking, and inactivity contribute to the development of more serious metabolic risk factors including atherogenic dyslipidemia and elevated plasma glucose that commonly manifest as a pro-thrombotic and pro-inflammatory state [6]. The National Cholesterol Education Program's Adult Treatment Panel III report (ATP III) identified 5 components that contribute to MetS: abdominal obesity (measured as waist circumference, men >102 cm; women >88 cm), triglycerides (≥150 mg/dL), HDL cholesterol (men <40 mg/dL, women <50 mg/dL), blood pressure (>120/>85 mmHg), and fasting glucose (≥110 mg/dL). When three of the five characteristics are present, a diagnosis of Metabolic Syndrome is confirmed [88]. The diagnosis of MetS was developed as a clinical measure for identifying individuals at risk for CVD and T2DM [89]. By concentrating on clinical causes such as obesity, hypertension, and dyslipidemia, it allows for the prevention of CVD and T2DM before the conditions are fully developed [3, 89].

In a population free of prevalent coronary heart disease (CHD), stroke, and T2DM, men and women with metabolic syndrome are ~1.5 and 2 times more likely to develop CHD than control subjects even after adjusting for age, smoking, LDL cholesterol, and race [90]. Findings from the Third National Health and Nutrition Examination Survey

(NHANES III) using 2000 census data show that about 47 million US residents have metabolic syndrome, an age-adjusted prevalence of nearly 24% [91]. As of 2006, the prevalence of MetS had increased to 34% and, considering this data is nearly a decade old, the current estimation is likely significantly higher [92]. The American Heart Association/National Heart, Lung, and Blood Institute recognize that insulin resistance and abdominal obesity are the predominant risk factors for Metabolic Syndrome [6].

It has been suggested that obesity is the common mechanism linking metabolic syndrome. Abdominal obesity correlates strongly with insulin resistance and an unusually high release of non-esterified fatty acids from adipose tissue [93]. The resultant lipid accumulation at the liver and musculature further predisposes to both insulin resistance and dyslipidemia [94, 95]. Adipose tissue produces pro- and anti-inflammatory cytokines and other bioactive substances that appear to induce or maintain an inflammatory state, including activation of intracellular pathways that promote obesity and T2DM. Lipid accumulation within adipocytes initiates cellular stress and activation of cell signaling pathways associated with inflammation including NF-KB. The NF-KB pathway regulates the proteins associated with the pathogenesis of both atherosclerosis and insulin resistance suggesting that obesity induced inflammation promotes both pathologies through common mechanisms and more importantly might be treated similarly [96]. Additionally, decreases in inflammation may down-regulate production of proteins involved in insulin resistance, T2DM, and CVD. Clearly, the increased adiposity associated with obesity maintains a state of chronic, low-grade inflammation that likely exacerbates MetS [46, 97].

ATHEROSCLEROSIS AND INSULIN RESISTANCE

As mentioned above, two predominant risk factors for MetS are atherosclerosis and insulin resistance. Insulin resistance, a recognized hallmark of abnormal metabolic function, is characterized by excessive insulin secretion and/or impaired insulin signaling [6, 7]. Atherosclerosis is a form of chronic inflammation of the blood vessel walls that can lead to ischemia, end-organ damage, stroke, or myocardial infarction. Both the cause and effect of these pathologies are intricately connected with postprandial metabolism. While the mechanisms may differ, increasing severity of both insulin resistance and atherosclerosis contribute to the development of MetS and, eventually, CVD and T2DM.

Atherosclerosis

Atherosclerosis is responsible for one third of deaths world-wide and 42% of deaths in the U.S. [69, 96]. Atherosclerosis is the central pathological mechanism of vascular diseases including coronary artery disease (CAD), stroke, and peripheral vascular disease. The working definition of atherosclerosis is "a systemic dysfunctional endothelial, focal occurring, chronic inflammatory, fibro-proliferative, pro-thrombotic, angiogenic, multifactorial disease of the arterial intima caused by the retention of modified low-density lipoproteins, hemodynamic, and reductive-oxidative stress" [98].

There is consensus in the literature that T2DM and CVD are interrelated pathologies. Compared with non-diabetic subjects, those with diabetes have a two- to four-fold increase in CAD and a greater extent of coronary ischemia [99]. In patients undergoing diagnostic coronary angiography, T2DM patients display more severe and diffuse coronary atherosclerosis [100]. In a population-based autopsy study, coronary atherosclerosis was

detected in 49% of diabetic decedents compared with 33% of those without diabetes [101]. These findings highlight the interrelated nature of CVD and T2DM, suggesting that they may be treated through similar mechanisms.

Pathophysiology of Atherosclerosis

Atherosclerosis is considered a form of chronic inflammation resulting from a complex interplay between modified lipoproteins, immune cells, and the normal cellular elements of the arterial wall [1, 8, 102]. Pathogenically, it is a decades-long expansion of the arterial intima, the area between endothelium and underlying smooth muscle cells. The initial insult is the translocation of an LDL molecule into the sub-endothelial space where it becomes oxidized (becoming oxLDL). This initiates inflammation and immune responses, including the infiltration of T-cells, monocytes, and inflammatory cytokines [9]. The resulting complex lesions, or plaques, that protrude into the arterial lumen, are commonly known as fatty streaks. Eventually, monocytes and macrophages infiltrate the sub-endothelial space to phagocytize oxLDL and form foam cells that secrete further inflammatory markers [8, 103, 104]. Eventual plaque rupture and thrombosis result in acute clinical complications such as myocardial infarction and stroke [105]. While genetic and environmental factors have been identified in epidemiological studies, elevated serum cholesterols are sufficient to drive the pathogenesis of atherosclerosis independent of other known risk factors.

Atherosclerosis and Vasculature

The endothelium is the primary barrier against atherosclerosis. Lining the internal lumen of all vasculature, the endothelium is metabolically active and acts as the interface between blood and vascular smooth muscle cells. Through autocrine, paracrine, and endocrine mechanisms, the endothelium regulates vascular tone and structure while exerting anticoagulant, antiplatelet, and fibrinolytic effects [106, 107]. A healthy endothelium maintains vascular homeostasis by keeping atherogenesis in check in part by the contributions of nitric oxide (NO) [108] .

Within endothelial cells, NO is synthesized from L-arginine by the enzyme eNOS. Shear stress within the vessel stimulates NO release from the arterial wall into the lumen and acts as a vasodilator to increase brachial artery diameter [11]. In addition to its role as a vasodilator, NO prevents thrombus formation and vasospasm as an inhibitor of platelet aggregation and smooth muscle cell proliferation, respectively [108]. Damage to the endothelium initiates the promotion and exacerbation of atherosclerosis including increased endothelial permeability, platelet aggregation, leukocyte adhesion, and cytokine production [1]. Eventually, the endothelium undergoes phenotypical alterations to this non-adaptive state known as “endothelial dysfunction” which manifests due to a loss or dysregulation of homeostatic mechanisms. Endothelial dysfunction is defined as the failure of the vascular endothelium to achieve its normal role of vasodilation and preservation of vascular homeostasis [109].

Endothelial dysfunction is an early marker for atherosclerosis and presents before angiographic or ultrasound evidence of plaques is detected [10]. It is characterized by

increased adhesion molecule expression, synthesis of pro-inflammatory or pro-thrombotic factors, oxidative stress, and abnormal modulation of vascular tone. Moreover, the balance between vasodilation and constriction is upset [110, 111]. Specifically, impaired vasodilation from decreased production or activity of NO is one of the earliest signs of atherosclerosis [112]. During the formation of an atherosclerotic plaque, oxLDL is retained within the intima of arteries, which activates endothelial cells and upregulates the expression of adhesion molecules and the secretion of chemokines [8, 103]. Normally, NO acts to prevent the oxidation of LDL [113]. However, the presence of oxLDL within the sub-endothelial space decreases NO production [114]. With the loss of NO production or activity, contributors to atherosclerosis are initiated including vasoconstriction, smooth muscle cells proliferation, and oxidative stress [115, 116]. Clearly, even the initial insult of atherosclerosis instigates endothelial damage and as the pathogenesis progresses, so too does the severity of vascular remodeling and subsequent dysfunction.

Both plasma and macrophage content of oxLDL in coronary plaques correlate with the severity of acute coronary syndrome [117]. Studies using both physiological (via flow-mediated dilation) and pharmacological (via acetylcholine) measures found endothelial dysfunction in patients that presented with risk factors for, but not ultrasound or angiographic evidence of, CAD [118]. Present at both conduit and microvascular levels, this confirms that endothelial dysfunction is present in the preclinical stages of atherosclerosis [11, 12]. In fact, coronary endothelial vasodilator dysfunction is an independent predictor of atherosclerotic progression and CV events even after adjusting for conventional risk factors [119]. In patients with and without CAD, acute cardiovascular

events are predicted by epicardial and microvascular coronary endothelial dysfunction [120].

Insulin

The hormone insulin plays a central role in the regulation of glucose and triglyceride metabolism and is largely responsible for the maintenance of normal postprandial glucose concentration. With normal insulin sensitivity, an ingested carbohydrate load is shortly followed by a transient rise in plasma glucose and a proportional rise in insulin to maintain normal glucose homeostasis. Insulin reduces this postprandial hyperglycemia by inhibiting hepatic glucose output and stimulating both glucose uptake and glycogen synthesis in skeletal muscle. Insulin also plays an important role in triglyceride metabolism by stimulating fatty-acid uptake and storage in muscle and adipose tissue. Thus, the net effect of insulin is the attenuation of the rise in postprandial glycemia and triglyceridemia.

Insulin and Vasculature

In addition to its metabolic effects, insulin plays a critical role in the regulation of blood flow in the vasculature. The primary mechanism by which insulin exerts its vascular effects is via endothelial cell production of NO. NO production induces vasorelaxation and increases capillary recruitment and blood flow in target tissues, specifically skeletal muscle. The end effect is augmented glucose disposal. Insulin infusion at higher physiological concentrations with a euglycemic clamp elicits a dose-dependent increase in skeletal muscle blood flow [121].

Insulin differentially modulates the vasculature tree. Initially, dilation of the terminal arterioles increases perfusion and capillary recruitment, followed by relaxation of the larger resistance vessels that increases overall limb blood flow. Animal and human studies have confirmed that this increased blood flow in skeletal muscle is due to the vasodilatory effects of NO production [122]. The vascular protective effects of insulin, including vasodilation, inhibition of vascular smooth muscle cell migration and proliferation, attenuating inflammatory cell infiltration, and inhibition of platelet aggregation, are all mediated by NO production in the endothelium [110].

Incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are released from the small intestines upon the absorption of glucose. These hormones inhibit glucagon and stimulate insulin release, accounting for nearly half the rise in postprandial plasma insulin [123]. With normal insulin sensitivity, insulin, GIP, and GLP-1 inhibit hepatic glucose output, stimulate skeletal muscle glucose uptake, and facilitate intramuscular glycogen synthesis. The end result is a reduction in postprandial hyperglycemia.

Insulin Resistance Pathophysiology

Insulin resistance, characterized by excessive insulin secretion and/or impaired insulin signaling, is an abnormal metabolic function that may facilitate or prolong elevated postprandial plasma glucose and triglycerides and necessitates elevated insulin secretion. Abnormal insulin response leads to metabolic imbalances, including hyperglycemia, hyperinsulinemia, and hypertriglyceridemia that are maintained in a perpetual cycle of metabolic dysfunction. This imbalance also affects the release of GIP and GLP-1. In

individuals with diabetes, the effects of GIP are impaired or lost while those of GLP-1 are unaffected, which could contribute to decreased insulin production and prolonged states of postprandial hyperglycemia and hypertriglyceridemia. [124].

The hypersecretion of insulin, such as in states of insulin resistance or caloric excess, inhibits the ability of insulin to attenuate postprandial hyperglycemia and hypertriglyceridemia. However, this is problematic because insulin is an inhibitor of fatty-acid oxidation [125]. Thus, the low resting fat oxidation increases the risk of weight gain and obesity and is a determinant of the elevation in postprandial triglyceridemia [126, 127]. Insulin resistance has been implicated as an indirect cause of atherogenesis by promoting the development of dyslipidemia. Early in the pathogenesis of insulin resistance, free fatty acids increase due to the loss of the suppressive effects of insulin on lipolysis in adipocytes. Moreover, when there is increased fatty-acid uptake into skeletal muscle, as seen in obesity or insulin resistance, the absence of a relative increase in fat oxidation or storage may result in the accumulation of fatty-acid intermediates which further impairs insulin signaling [128, 129].

Insulin Resistance and Vasculature

The vasoregulatory action of insulin on endothelial cells stimulates the production of vasoconstrictors and vasodilators. Normally, insulin is protective and vasodilatory effects are mediated by NO production [130, 131]. Insulin resistance plays a major role in the onset of endothelial dysfunction by inducing a loss of vascular reactivity, impaired normal vasomotion, and reducing blood flow. These impairments, and the associated endothelial dysfunction, precede atherosclerosis [132-134]. It has been shown that

endothelial dysfunction is closely correlated with the presence of insulin resistance and that the effect of insulin on atherosclerosis is primarily mediated through its effect on other CVD risk factors, including dyslipidemia and hypertension rather than by a direct effect of insulin [134-136].

In addition to the metabolic disturbance evident in insulin-resistant states, the vascular effects of the hormone are also impaired. Insulin-resistant rodent models have shown decreased eNOS activity, defective insulin signaling in vascular tissues, and impaired insulin action on endothelial cells [130, 137]. In humans, the simultaneous metabolic and vascular impairments to insulin resistance have been confirmed. The induction of acute insulin resistance by the infusion of free fatty acids causes acute insulin resistance and is characterized by a simultaneous decrease in glucose uptake and endothelium-dependent vasodilation [22]. The impairment of vascular responses is proportional to the degree of insulin resistance [36]. It is known that skeletal muscle vasodilation in response to insulin infusion is reduced in obese subjects and that differences in blood flow rates are most relevant within physiological insulin ranges [36, 138]. In patients with T2DM, insulin therapy improves endothelial function [139]. The finding that pharmacological interventions reverse endothelial defects supports the close relationship between insulin resistance and endothelial dysfunction. It is widely accepted that multiple independent and interdependent mechanisms induce a circle of dysfunction between insulin resistance and vascular dysfunction that explains the association between metabolic and vascular diseases [121].

POSTPRANDIAL METABOLISM AND ACUTE VASCULAR DYSFUNCTION

In the past, clinical evaluation of hyperglycemia and hypertriglyceridemia as a measure of CVD and mortality has utilized fasting measures. However, this likely represents the nadir of daily blood glucose and triglycerides, respectively. Instead, it is becoming increasingly well accepted that atherosclerosis is a postprandial phenomenon [37]. Most humans eat every four to five hours, but it requires upwards of eight hours for complete clearance of glucose and triglycerides from the blood stream. This suggests that most humans spend the majority of their day in a postprandial state. Therefore, postprandial measures represent a more robust indication of daily plasma glucose and triglyceride exposure and a truer reflection of the associated risks [13, 15, 17, 18, 21, 140, 141].

Hyperglycemia

Precise matching of glucose utilization with endogenous glucose production and dietary glucose delivery is required for maintenance of normal plasma glucose concentrations. In a fasting state, plasma glucose is relatively stable, indicating that production and utilization rates are equal [142]. After a meal, glucose absorption increases plasma glucose concentrations more than twice the rate of endogenous glucose production. Postprandial hyperglycemia depends on timing, quantity, and composition of the meal, the total amount of carbohydrate, the rate and degree of glucose absorption, the secretion of insulin, and inhibition of glucagon [143].

Epidemiological studies have consistently shown that serum glucose levels two hours after an oral glucose challenge are significant predictors of CV risk [38]. These findings have been confirmed by two meta-analyses that have found that a 2-hour glucose challenge predicts CHD [13, 144]. Postprandial hyperglycemia has been identified as a possible independent risk factor for CVD by its association with intima-media carotid thickening [145]. Finally, serum glucose levels are associated with infarction prognosis in non-diabetic subjects [146], and postprandial glycemia is an independent risk factor for CHD in patients with T2DM [21].

An intervention study using subjects with impaired glucose tolerance determined whether an intervention to limit postprandial hyperglycemia would reduce the risk of CVD. Acarbose, an a-glucosidase inhibitor that specifically reduces postprandial hyperglycemia, was associated with a 34% reduction in risk of developing new cases of hypertension and a 49% risk reduction in CV events [147]. In addition, acarbose treatment is associated with a reduction in intima-media thickness in T2DM patients [148] as well as with a significant reduction in CV events independent of other risk factors [149]. In the progression of atherosclerosis, an LDL molecule enters the sub-endothelial space and becomes oxidized. LDL oxidation increases acutely after meals in T2DM further contributing to augment the degree of hyperglycemia, [150, 151]. Clearly, attenuating postprandial hyperglycemia may positively affect CVD development.

Hyperglycemia and Vasculature

Acute hyperglycemia, independent of insulin levels, significantly attenuates forearm endothelium dependent, but not independent, blood flow in healthy humans [23]. In both diabetic and healthy subjects, hyperglycemic spikes have been shown to induce endothelial dysfunction. It is purported that these effects are linked with a reduced production or bioavailability of NO since the hyperglycemia-induced endothelial dysfunction is counterbalanced by arginine [152]. The rapid decrease in flow-mediated dilation (FMD), a measure of endothelial dependent vasodilation, is inversely correlated with the magnitude of postprandial hyperglycemia in T2DM subjects [153].

Thrombin is a molecule involved in the coagulation cascade, alterations of which are linked to thrombosis. Postprandial hyperglycemia causes an overproduction of thrombin which has been shown to be strictly dependent on blood glucose levels [154]. Further, adhesion molecules regulate the interaction between endothelium and leukocytes and intracellular adhesion molecule-1 (ICAM-1), specifically, increases in subjects with diabetes and/or vascular disease. Postprandial hyperglycemia has been shown to be a sufficient stimulus for increased circulating levels of ICAM-1, thus activating one of the first stages of atherosclerosis [155, 156]. Finally, acute hyperglycemia has been shown to increase the production of inflammatory cytokines, including IL-6, TNF-a, and IL-18 [157, 158].

Hypertriglyceridemia

Lipoproteins are comprised of lipids and one or more apolipoproteins, the particle that interacts with cell receptors and enzymes [159]. Various lipoproteins are classified according to density and are involved in several metabolic processes: very low density lipoproteins (vLDL) and chylomicrons transport triglycerides from endogenous sources and exogenous sources, respectively, to peripheral tissues and HDL carries cholesterol from the periphery to the liver [160]. Lipoprotein lipase, present on the luminal side of endothelial cells, is responsible for the hydrolysis of triglycerides into glycerol and free fatty acids. After consumption of a high fat meal, chylomicrons carry triglyceride through the vasculature where fatty acids are liberated by lipoprotein lipase and taken up by surrounding adipose and muscle cells [161]. Triglyceride removal is dependent on LPL activity and the tissues' need for lipids [162]. In a postprandial state, LPL availability becomes limited due to competition for binding sites and causes triglyceride rich lipoproteins, including chylomicrons, vLDL, and remnants, to accumulate [19, 163].

In hypercaloric diets such as with Metabolic Syndrome, obesity, and T2DM, there is a chronic state of LPL competition and increased free fatty acids. The magnitude of postprandial plasma lipid increase is directly proportional to the fat content in meals [20, 72, 73]. After a high fat meal, plasma triglycerides increase, peaking around four hours and returning to fasting levels around eight hours. Because a fatty meal increases plasma lipids, multiple high fat meals throughout the day results in prolonged presence of elevated plasma triglyceride.

Increased plasma triglyceride results in reduced HDL and increased small, dense LDL. This contributes to an increased susceptibility to oxidation and a perpetual cycle of hypertriglyceridemia and atherosclerosis [164]. Plasma triglycerides are important determinants of plasma cholesterol metabolism, but evidence indicates that postprandial triglycerides are better predictors of atherosclerosis and coronary artery disease than fasting levels [14-17, 20, 140, 165]. The classification based on the magnitude of postprandial hypertriglyceridemia demonstrates a 68% accuracy in detecting the presence of CVD [166]. Postprandial triglyceride concentrations are associated with carotid artery wall thickness and represent an independent risk factor for atherosclerosis [141, 167, 168]. Dyslipidemia is a recognized risk factor for CVD in diabetes, and postprandial hyperlipidemia contributes to this risk [169, 170]. Every 1.13 mmol/L increase in postprandial hypertriglyceridemia is associated with an increase in relative risk of 1.4 for a myocardial infarct [171].

Hypertriglyceridemia and Vasculature

Endothelial dysfunction is present in states of hypertriglyceridemia and hypercholesterolemia and is attributed in large part to reduced bioavailability of NO [70]. Though the exact mechanism for the reduced NO bioavailability is unknown, it could include any number of impairments to receptors, L-arginine use, concentration and activity of eNOS, release of NO, and NO diffusion [107]. Endothelial dysfunction is related to postprandial serum triglycerides after a high fat meal in middle aged men and women [71]. A study comparing a low and high fat meal found that the high fat meal transiently impaired

endothelial function and there was a negative correlation between mean triglyceride changes and endothelial dysfunction [172]. Moreover, the non-physiological infusion of a triglyceride emulsion induced a loss of vascular reactivity mediated by both endothelium-dependent and endothelium-independent mechanisms [173]. Even in young, healthy men, postprandial triglyceride levels are closely associated with impaired brachial artery FMD after a high fat meal [174].

Atherosclerosis is dependent on LDL, remnant lipoprotein concentrations, and perturbations on the vessel wall that influence the rate of arterial lipoprotein retention. Thus, both hypertriglyceridemia and hypercholesterolemia promote atherosclerosis and the associated vascular dysfunction [19, 20]. Atherogenic hypertriglyceridemia is due to impaired uptake of cholesterol remnants, not defective triglyceride hydrolysis [20]. Acute hypertriglyceridemia, induced by a high fat meal, correlates negatively with changes in FMD, but positively with changes in leukocyte $O_2^{-\circ}$ production, a marker of oxidative stress. These changes were not seen with a low fat meal, indicating that acute hypertriglyceridemia causes endothelial dysfunction via at least a partial contribution from enhanced oxidant stress [175]. Hypercholesterolemia impairs the L-arginine pathway, through which NO is produced, by activating the angiotensin-II receptors to cause vasoconstriction and neurohumoral activation. This increases the release of reactive oxygen species thereby decreasing NO production and increasing vascular cell apoptosis and expression of adhesion molecules, chemotactic factors, and pro-inflammatory cytokines [104].

The relationship between postprandial metabolism, vascular dysfunction, and metabolic diseases has been fairly well established. Considering that for most individuals in modern society, a majority of time is spent in a postprandial state, identification of treatments that moderate or attenuate postprandial hyperglycemia and hypertriglyceridemia is needed. Lifestyle modifications, including dietary interventions, offer an affordable and easily implemented alternative to pharmacological interventions. The hallmark risk factors of MetS, atherosclerosis, and insulin resistance, are postprandial by nature and so relevant research for MetS, CVD, and T2DM must include nutrition interventions that assess metabolic function.

METABOLIC AND VASCULAR EFFECTS OF DAIRY

Bovine milk is comprised of approximately 87% water, 4-5% lactose, 3% protein, 3-4% fat and less than 1% of vitamins and minerals combined. Milk supplies 32 grams of protein per liter. Of the milk protein fraction, 20% is whey, a soluble protein, and 80% is casein, an insoluble protein. Both are considered high-quality proteins because they provide essential amino acids, are readily digested, and have high bioavailability. These two protein fractions differ in their amino acid profile. Whey protein is rich in branched chain amino acids (leucine, isoleucine, and valine) whereas casein is higher in histidine and phenylalanine. The fat fraction of milk, present as globules, is dependent on animal origin, stage of lactation, and feed-related factors. Typically, the fat found in milk is comprised of 98% triglycerides, of which 70% is saturated fatty acids and 30% is unsaturated fatty acids. Lactose, the carbohydrate found in milk, is a dissacharide sugar

comprised of galactose and glucose. The glycemic index of lactose is 45 compared with the reference 100 for glucose. In addition to the primary macronutrient composition, dairy products have a specific micronutrient composition including calcium, magnesium, and vitamin D [63].

Epidemiological and prospective studies on dairy

Epidemiological and prospective studies have found chronically high consumption of dairy products is associated with a reduced risk of diabetes and CVD. Dairy intake is negatively associated with the risk of developing diabetes: individuals in the top quintile of dairy intake have a reduced risk of diabetes compared with those in the lowest quintile. Specifically, for each daily serving of dairy consumed there was a 9% and 4% decrease in risk of diabetes in men and women, respectively [24-26]. Further, milk consumption is inversely associated with the overall risk of CVD and stroke [74, 75]. There is a 15% lower relative risk for all-cause mortality and an 8% lower overall relative risk of ischemic heart disease with high dairy consumption [49]. Similarly, there is an inverse association between dairy intake and MetS development in healthy, overweight, and obese individuals [27, 28]. Compared with less than ten servings/week, consuming more than thirty-five servings/week decreased the odds of developing MetS by 72% and each daily serving of dairy reduces the risk of MetS by 21% [27].

These studies examining the relationship between dairy and MetS, CVD, and T2DM indicate that higher consumption of dairy elicits favorable effects on metabolic health. Since the pathologies are metabolic in origin and develop in a postprandial state,

investigating nutrition interventions are necessary to elucidate the potential physiological mechanisms regulating these relationships.

Dairy and Glycemia

Long-term Intervention Studies

Dietary intervention studies investigating the effects of milk or dairy products and glucose response are few; however, results from published trials indicate a range of effects, likely due to the different types of dairy employed. In a six-week randomized cross-over trial, subjects replaced 13% of their daily energy intake with either butter or cheese of equivalent fat content. Compared with the butter trial, fasting blood glucose increased after the cheese intervention. Authors admit this finding was unexpected and offer no explanation, but offer the evidence that insulin resistance values were no different between trials [176]. An eight-week clinical trial investigating the effects of low-fat dairy intake on overweight and obese men found no effect on fasting glucose concentration [177]. However, a 6-week randomized control trial found decreased plasma glucose concentrations with high dairy consumption compared with a control food in obese women [178]. Fasting blood glucose concentration did not change within or between trials in hypertensive subjects who consumed 4+ servings of low-fat dairy or eliminated all dairy for 4 weeks [179]. The discrepancy between the above mentioned studies are likely attributed to metabolic health of the subject population; in the previous study, subjects demonstrated elevated fasting glucose consistent with pre-diabetes (5.98-6.08 nmol/L)

whereas in the latter study, subjects exhibited normal fasting glucose levels (5.27-5.44 mmol/L). While the long-term effects of dairy interventions are relevant to the treatment and prevention of metabolic diseases, postprandial studies provide descriptive information regarding mechanisms involved.

Postprandial Intervention Studies

The effects of dairy on postprandial metabolism indicate that milk elicits favorable effects on glucose metabolism both alone or with a meal. In a well-designed randomized cross-over study, whole milk (a control), a beverage based on equivalent milk macronutrients, complete milk protein (16g), lactose (24 g), or milk fat (16g) were compared for metabolic response. Whole and simulated milk lowered blood glucose more than predicted by the sum of the area under the curve (AUC) for individual components [180]. Low fat milk reduced pre and post-meal peak blood glucose and post-meal glucose AUC compared with water, soy beverage, 1% chocolate milk, orange juice, or a cow milk-based infant formula [51, 52].

The majority of intervention studies examining postprandial metabolism and dairy employ milk derived proteins, specifically the now familiar supplement whey protein. The incremental area under the curve (iAUC) for glucose decreased in a dose dependent manner with the highest dose of whey protein supplement (20 g) having a significantly greater effect than lower doses on postprandial hyperglycemia from a 50g glucose drink [181]. Similarly, increasing doses of whey protein (10-40 g) pre-meal reduced post-meal blood glucose and insulin AUC in a dose dependent manner [53]. The combination of whey

protein and carbohydrate intake resulted in higher plasma insulin and reduced plasma glucose compared with those consuming carbohydrate alone [55]. A high glycemic meal for breakfast and lunch with whey protein increased serum insulin at breakfast 31% and lunch 57% compared with meals without whey. Further, the consumption of whey decreased postprandial serum glucose by 21% compared with the test meal without whey [41]. Individuals with diabetes who consumed 50 g of whey or placebo with a high glycemic breakfast and found glucose levels were reduced 28% and insulin increased 105% after the protein preload. Interestingly, while not compared in head-to-head fashion, these authors point out that the decrease in glycemia was a larger reduction than that observed after different doses of a rapid-acting non-sulfonylurea insulin secretagogue (18%) [54, 182]. Clearly, whey protein consumption both in healthy and diabetic individuals attenuates the rise in postprandial glycemia when combined with a high carbohydrate load.

Dairy and Lipemia

Dyslipidemia is a significant risk factor for MetS, CVD, and T2DM and an increasing number of studies are examining the effects of long-term dairy consumption on the lipid profile. Of the total cholesterol pool, cholesterol carried by LDL is accepted as a better indicator of CVD risk due to the atherogenicity of LDL. Increased LDL is associated with increased consumption of SFA; however, a number of intervention studies using whole fat milk and other whole fat dairy products have not shown significant increases in LDL cholesterol [50, 183, 184]. Regarding the lipid profile, an increased proportion of small, dense LDL (sdLDL) particles represents a greater atherogenic risk than larger, less

dense LDL cholesterol molecules. In a cross-sectional study investigating healthy men, sdLDL particles were positively related to plasma triglycerides and fasting insulin levels and inversely to HDL. Individual fatty acids typically found in milk products were associated with fewer sdLDL particles suggesting milk's fatty acids are associated with a more favorable lipid profile [185].

Long-term Intervention Studies

In a 10-year longitudinal study, a higher intake of dairy saturated fat was associated with a lower CVD risk compared with a higher intake of meat saturated fat. Interestingly, substitution of 2% of energy from meat SD with energy from dairy saturated fat was associated with a 25% lower CVD risk. The authors acknowledge that this attenuation was likely attributed to other components of dairy such as calcium, magnesium, and/or bioactive peptides, but it could have been due to a difference in the relative proportions of different saturated fatty acids in meat and dairy [186]. However, a five-year prospective study of three hundred women demonstrated that total dairy, milk, yogurt, cottage cheese, and calcium were positively related to triglycerides and negatively to HDL cholesterol at baseline, but no association was found for any five year changes [187]. Healthy normocholesterolaemic males who consumed 20% of dietary energy as butter for twenty-one days showed no significant change in blood lipid or apolipoprotein profile [50].

Studies show conflicting results regarding milk protein's effects on lipid profiles. Whey protein isolate supplementation over three months significantly reduced fasting triglycerides, total cholesterol, and LDL cholesterol in overweight and obese adults [188].

A similarly designed study employing a malleable protein matrix (protein enriched yogurt) reduced fasting triglycerides, and this effect was more pronounced in subjects with elevated triglycerides at baseline [189]. However, three-month supplementation with whey protein during a weight regain study showed no effect on plasma lipids [190]. In a study examining the effects of lactotripeptide supplementation with or without exercise on vascular measures, there was no change in lipid panel including total cholesterol, LDL, HDL, or triglycerides in postmenopausal women after eight weeks of lactotripeptide supplementation [191]. Though the number of studies are limited, the available evidence indicates that milk proteins may have a beneficial effect in individuals with poorer metabolic health.

Postprandial Intervention Studies

Postprandial triglyceridemia is strongly influenced by the composition of the meal, including the quality and quantity of fat. To date, there are few studies examining the role of complete dairy products on postprandial metabolism [32, 33]. The majority of dairy and lipemia studies have examined the effects of milk-derived proteins on postprandial metabolism. Compared with control, the postprandial appearance of triglycerides was decreased 21% and 27% when a meal was consumed with whey and casein, respectively [77]. There was no difference between four milk-derived proteins on postprandial plasma triglycerides during an eight-hour high fat test [76]. Postprandial apolipoprotein B-48 (apo B-48) response to a high fat meal was significantly reduced when consumed with 60 g of whey protein compared with an equivalent dose of casein protein, independent of medium

chain SFAs. This reduced apo-B-48 is indicative of a reduced number of chylomicron particles within the blood stream suggesting the potential of dairy to reduce CVD risk associated with a high fat meal [192]. While acute studies examining the effects of milk proteins on lipid levels are spare, they agree in their findings of improved postprandial lipemia with addition of milk-derived proteins to a meal.

Vascular Function and Dairy

Long-term studies examining the effects of dairy in general suggest favorable effects on vascular function. In a two-month study examining the effects of lactotripeptide supplementation with or without exercise on cardiovascular health in post-menopausal women, lactotripeptide supplementation, alone and with concomitant aerobic exercise, reduced blood pressure and improved flow-mediated dilation and arterial compliance [191, 193]. A one-month study supplementing hypertensive subjects with specially formulated whey protein blend showed improvements in vascular reactivity as measured by flow-mediated dilation, up 3% compared with baseline [194]. Compared with a control group, subjects who consumed both whey and casein with concomitant exercise demonstrated favorable changes in augmentation index and pulse wave velocity [195]. However, a study comparing intact whey, casein, and semi-skimmed milk in overweight adolescents found improvements in blood pressure, but failed to find significant changes in vascular measures of arterial stiffness, including augmentation index and pulse wave velocity [196]. However, arterial stiffness is an age-related disease so likely the subject population exhibited near

optimal values for augmentation index and pulse wave velocity. Thus the basement effect would have limited the potential benefits of dairy.

Few studies have been conducted investigating vascular effects during the postprandial state with milk or milk proteins. Most of those studies employed measures of arterial stiffness as the vascular measure, specifically pulse wave velocity (PWV). PWV is a useful clinical measure as it is an independent predictor of CVD. However, significant changes in PWV occur primarily with chronic interventions making it a less-than-ideal tool for postprandial studies. Every study to date to evaluate the postprandial effects of milk proteins on arterial stiffness found no effects [77, 197, 198]. Flow-mediated dilation, a measure of endothelial-dependent vasodilation, may change acutely as it is depressed in hyperglycemic, hyperlipidemic, and hyperinsulinemic states [199]. Interestingly, in mild hypertensive, overweight subjects, FMD improved 4.3% at 120 minutes with a whey protein derivative compared with placebo [68]. There is no difference in NO dependent vasodilation in the microcirculation between milk and rice beverage control [200]. However, low-fat milk maintains conduit vessel vascular endothelial function in adults with MetS by limiting postprandial hyperglycemia when compared with rice milk [67].

The acute vascular effects of dairy in a postprandial setting have been minimally investigated, but the overall findings suggest a favorable response. FMD is a measure of endothelial-dependent vasodilation and improvements in FMD imply increased NO production and its protective effects. The potential for dairy to limit postprandial impairments in FMD indicates significant therapeutic applications for at-risk individuals.

However, the investigations into the vascular effects of dairy are still in an early stage and mechanisms of action have yet to be fully determined.

Mechanisms of improvement

Protein

Clearly, the beneficial effects of milk as seen in cross-sectional and epidemiological studies are supported by long-term clinical trials and postprandial intervention studies. Regardless of the outcome, nearly all studies attribute the favorable metabolic effects of dairy, at least in part, to the insulinotropic effects of milk proteins. Indeed, compared with other protein sources, milk demonstrates a larger insulin response up to 240 minutes post-meal [30].

The suggested mechanisms for improved glucose regulation include a protein-induced increase in serum insulin when milk products are added to a meal high in carbohydrates. The high amino content, branch chain amino acids (BCAA) in particular, may modulate glucose levels by increasing postprandial insulin secretion. Interestingly, whey hydrolysate elicits higher insulin responses compared with whole milk [201] and intact whey protein [202]. Likely, this is due to the partially-digested proteins in whey hydrolysate eliciting a faster release of insulin. Whey protein has been shown to increase GIP, which triggers the release of insulin from pancreatic beta cells [203]. Increased insulin secretion is especially important in T2DM as diabetic individuals typically experience a decreased insulin response to carbohydrates.

As with glycemic control, the lipid lowering effects of dairy are primarily attributed to the insulinotropic effects of dairy. Increased insulin during postprandial lipemia inhibits hormone-sensitive lipase and the release of hepatic FFA and stimulates lipoprotein lipase which hydrolyzes triglycerides for metabolism and storage [79].

Fat

While the protein content of dairy is typically the primary constituent associated with reductions in glycemia, evidence is emerging that points to dairy's fat content as a potential contributor. In a cross-sectional study of more than 10,000 Brazilian adults, the intake of total dairy was inversely association with fasting glucose and postprandial glucose after adjusting for covariates. Interestingly, myristic acid (14:0), a long chain saturated fatty acid found in dairy foods, was the only apparent nutrient to mediate the association between dairy intake and glycemia indicating this SFA may play a role in improving glucose homeostasis [204]. This finding is supported by EPIC –InterAct case-cohort study, which showed a positive relationship between myristic, palmitic, and stearic acid concentrations in plasma phospholipids and diabetes risk [205]. However, it is important to note that the glycemic control exerted by dairy products is evident in non-fat and low-fat dairy products. Thus, the potential role of milk fats in regulating glycemia is likely in addition to other mechanisms, such as the insulinotropic effects of proteins.

Vitamins and minerals

In addition to the potential mechanisms of protein and fat in altering metabolism, dairy also contains a number of bioactive compounds with functional properties. The

combined vitamin and mineral content comprises less than 1% of milk, but this rather small volume suggests significant metabolic effects. A systematic review and meta-analysis that examined the role of vitamin D and calcium in T2DM found associations between low vitamin D status, calcium, or dairy intake and prevalence for T2DM and MetS. Additionally, there were inverse associations with both T2DM and MetS for the highest vs. lowest dairy intake. Evidence from vitamin D and/or calcium supplementation suggest that they may play an important role in preventing T2DM in populations at high risk, including glucose intolerance [206].

The leading food source of vitamin D in the American diet is fortified cow milk, which contains ~100 IU/8 oz [207]. Evidence indicates that vitamin D may beneficially mediate MetS risk factors [208]. In fact, a recent review reported that serum vitamin D status is inversely related to MetS and a separate meta-analysis of eight cross-sectional studies found that higher serum vitamin D levels reduced the prevalence of MetS nearly 50% compared with lower levels [81, 209].

The suggested mechanisms for the effect include reduced dyslipidemia and increased insulin production [81]. NHANES-III data show that plasma triglyceride levels are lower in individuals with >92.5 nmol/L of vitamin D compared with those with <62.5 nmol/L [210]. Additionally, several intervention studies have shown improved glycemic responses with vitamin D treatment, but these effects may be specific to individuals who were deficient at baseline or those who had preexisting metabolic disorders [65, 211, 212].

The relationship between higher dairy intake and reduced risk of stroke, all-cause mortality, T2DM, and ischemic heart disease, compared with lower total dairy intake has

not been fully elucidated, but it has been proposed that the high calcium content and lipid fractions in dairy benefits the serum lipid profile. Nearly half of the calcium in the American diet is from dairy [207]. Several randomized control trials have shown beneficial effects from calcium supplementation on plasma lipids [213-215]. Compared with consuming their standard diet, participants who consumed 2,200 mg/d of calcium from fortified foods for 10 days demonstrated decreased total cholesterol and LDL [213]. Serum cholesterol has been reduced by 15.4 mg/dL and triglycerides reduced 32.2 mg/dL with calcium supplementation (900 mg/day). The proposed physiological mechanism is that calcium binding to saturated fatty acids within the intestines forms insoluble soaps that are excreted in the feces. Healthy men consuming 1 L of milk or yogurt daily containing 1,200 mg calcium compared with placebo demonstrated increased fecal fatty acid and bile acid excretion during dairy intake. This resulted in lower fat absorption compared with the placebo [82].

Magnesium has also been proposed as a significant mediator in postprandial metabolism. Upwards of 10% of the magnesium in the American diet comes from dairy [63]. Data from epidemiological studies has consistently demonstrated an inverse relationship between magnesium and risk for incident insulin dependent and non-insulin dependent diabetes [216-218]. Clinical trials have demonstrated that oral magnesium supplementation has improved insulin sensitivity, glucose homeostasis, and HbA1c levels in diabetic patients [219]. Moreover, a meta-analysis of randomized control trials found 12 weeks of supplementing with magnesium significantly lowered fasting serum glucose in T2DM patients (186). The mechanism is attributed to improved insulin elicited glucose

uptake because magnesium is essential for optimal coupling and signaling through the insulin receptor. Regarding dyslipidemia, magnesium has been shown to reduce serum triglycerides, apolipoprotein B, LDL, total cholesterol, and to increase HDL in patients with ischemic heart disease [83, 220, 221]. These effects have been attributed to involvement of modifications of several enzymes intricately linked with lipid metabolism [84].

Dairy's micronutrient profile offers significant potential for favorable effects on the cardiometabolic profile. As with the insulinotropic effects of milk proteins, evidence suggests that these micronutrients offer their greatest benefits to individuals who are either deficient in the respective vitamin or mineral or who are farther along the spectrum of disease progression. However, their mechanisms of action have yet to be fully described as no study has yet to examine the role of dairy's micronutrients in a controlled intervention trial.

APPENDIX A: 3-DAY FOOD LOG

3-Day Food Intake Record Instructions

1. Record day of the week and date for everything you eat and drink for three days (two week days & one weekend day) prior to arriving at your appointment.
2. Include the time, amount and type of food/beverage consumed. Provide as much detail as possible, including brand names when available. For example, instead of recording "cereal with milk", record "1.5 cups Kashi GoLean cereal with 6 oz low-fat milk". Instead of "1 slice wheat toast with jam", record "1 slice Orowheat 100% whole-wheat toast with 1tsp Smucker's low-sugar strawberry preserves". See sample food log for more examples.
3. For combination foods such as chili, soup, casseroles, sandwiches, list all items in the food and amounts of each item.
4. For dairy products (milk, cheese, yogurt, etc) record whether, regular (whole), lowfat (1%), reduced fat (2%), or nonfat (skim).
5. Include sweeteners (sugar, honey, syrup, etc) and fats (cream, half&half, milk, etc) added to coffee, tea, etc; as well as spreads on breads and dressings on salads.
6. For meats, indicate type (ground, sirloin, etc) and % lean, if known.

Sample 3 Day Food Intake	**Day of Week:**		**Date:**
Time	***Amount***	***Brand***	***Food/Beverage***
8am	**8 oz**		**Nonfat milk (in cereal)**
	12 oz		**Black coffee**
	1 Tsp		**Sugar in coffee**
	1.5 Cups	**Nature's Path**	**Heritage Heirloom Whole Grains Cereal**
	1 T	**Sun-Maid**	**Fruit bits**
	1 medium		**Cara Cara navel orange**
12pm	**1.3 Cups**	**Homemade**	**Chili: ½ Cup 70% lean ground beef, 1 T onion, 2 T garbanzo beans, 2T black beans, 2 T red sweet pepper**
	3 T		**Grated cheddar/jack cheese, regular**
	½ Cup		**Fresh strawberries**
	½ Cup	**Stoneyfield**	**Lowfat vanilla yogurt**
	2 T		**Raw almonds**
3pm	**1**	**Cliff**	**Chocolate Builder's Bar**
6pm	**5 oz**		**Grilled chicken breast, skinless**
	¾ Cup		**Slaw: ¼ cup cabbage, ¼ grated carrots, ¼ broccoli, 1 tsp olive oil, 1 tsp cider vinegar**
	1 piece	**Kirkland Signature**	**Multigrain bread**
	½ tsp		**butter**
	¾ Cup		**Grilled vegetables: ¼ cup yellow squash, ¼ red pepper, ¼ cup eggplant**

Day ___

3 Day Food Intake	**Day of Week:**		**Date:**
Time	***Amount***	***Brand***	***Food/Beverage***

Day ___

3 Day Food Intake		**Day of Week:**	**Date:**
Time	***Amount***	***Brand***	***Food/Beverage***

Day ___

3 Day Food Intake		**Day of Week:**	**Date:**
Time	***Amount***	***Brand***	***Food/Beverage***

APPENDIX B: CONTROL DAY ACTIVITY AND DIET RECORD

Subject ID:_____ Pedometer #:_____

	Time	Steps			
Meal 1:	_____	_____	**Meal 1:**	_____	_____
Food #1			**Food #1**		
Food #2			**Food #2**		
Food #3			**Food #3**		
Food #4			**Food #4**		
Meal 2:	_____	_____	**Meal 2:**	_____	_____
Food #1			**Food #1**		
Food #2			**Food #2**		
Food #3			**Food #3**		
Food #4			**Food #4**		
Meal 3:	_____	_____	**Meal 3:**	_____	_____
Food #1			**Food #1**		
Food #2			**Food #2**		
Food #3			**Food #3**		
Food #4			**Food #4**		
Meal 4:	_____	_____	**Meal 4:**	_____	_____
Food #1			**Food #1**		
Food #2			**Food #2**		
Food #3			**Food #3**		
Food #4			**Food #4**		
Total Step			**Total Step**		
Count:	_____		**Count:**	_____	

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